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An assessment of discard mortality for two Alaskan crab species, Tanner crab (*Chionoecetes bairdi*) and snow crab (*C. opilio*), based on reflex impairment

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Abstract—Delayed mortality associated with discarded crabs and fishes has ordinarily been observed through tag and recovery studies or during prolonged holding in deck tanks, and there is need for a more efficient assessment method. *Chionoecetes bairdi* (Tanner crab) and *C. opilio* (snow crab) collected with bottom trawls in Bering Sea waters off Alaska were evaluated for reflexes and injuries and held onboard to track mortality. Presence or absence of six reflex actions was determined and combined to calculate a reflex impairment index for each species. Logistic regression revealed that reflex impairment provided an excellent predictor of delayed mortality in *C. opilio* (91% correct predictions). For *C. bairdi*, reflex impairment, along with injury score, resulted in 82.7% correct predictions of mortality, and reflex impairment alone resulted in 79.5% correct predictions. The relationships between reflex impairment score and mortality were independent of crab gender, size, and shell condition, and predicted mortality in crabs with no obvious external damage. These relationships provide substantial improvement over earlier predictors of mortality and will help to increase the scope and replication of fishing and handling experiments. The general approach of using reflex actions to predict mortality should be equally valuable for a wide range of crustacean species.

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Numerous species with economic and ecological significance are discarded from fishing operations because of harvest restrictions or low value compared with the target species or size groups. Although discarded catch can exceed that retained (Alverson et al., 1994; Witherell and Pautzke, 1997), discard mortality rates are rarely known. Mortality can occur on deck (immediate and observed mortality) or after the animal is released or escapes capture (delayed and unobserved mortality) (Stevens, 1990; Davis, 2002; Suuronen, 2005). Delayed mortality can result directly from physical injury and physiological stress or indirectly by an increased susceptibility to disease and predation, and through an inability to feed. Although a significant effort has been made to reduce unwanted bycatch through improvements in gear selectivity and handling procedures, unobserved discard mortality continues to be an important source of uncertainty for fishery management (Harrington et al., 2005; Broadhurst et al., 2006; Coggins et al., 2007).

Directed fisheries for *Chionoecetes* spp. and *Paralithodes camtschaticus* (red king crab) in the North Atlantic, North Pacific, and Bering Sea are prosecuted with baited pots, the only legal commercial gear for these crabs in the United States and Canada. Harvesting is restricted to males within strict size limits; consequently all females and undersized males are discarded. Crabs are also captured and discarded or escape from trawl gear. Given the difficulties of determining mortality for discarded crabs, fixed mortality rates have been assumed for specific fishing sectors in most fishery management models on the basis of a handful of experiments (see below). For example, fishery managers in Alaska have assumed fixed values of 20%, 50%, and 20%, respectively, for the discard mortality rates of *Chionoecetes bairdi* (southern

Tanner crab), *C. opilio* (snow crab) and *P. camtschaticus* in pot fisheries, and a fixed value of 80% for the discard mortality of both red king crab and snow crab in Bering Sea trawl fisheries (Siddeek, 2003).

Estimates for discard-related mortality in fishery species normally require experimental research whereby individuals or populations captured in different fishing operations or under different conditions are monitored for subsequent survival. Mortality can be evaluated through tag recovery studies, but this method requires handling large numbers of individuals, often for low returns. Holding fish or crabs in tanks or cages is a good method for direct evaluation of mortality in different fishing operations and handling, but experiments are often limited to relatively small numbers, short follow-up periods, and a few specific treatments or fishing variables.

A useful predictor for discard mortality may be crab condition. Blood and tissue chemistry (e.g., lactate, glucose, glycogen levels) have been used to evaluate stress in crustaceans (Crear and Forteath, 2001; Harris and Andrews, 2005; Ridgway et al., 2006). Recent experiments with marine fishes, however, show that chemical measures are often poor predictors of mortality because they typically reach peak values before any mortality occurs and because chemical measures often respond differently to physiological stress and physical injury (Davis et al., 2001, Davis and Schreck, 2005). Two other types of condition indicators have been explored for predicting mortality rates in crabs: injuries to the exoskeleton and behavioral impairments. In the first category, correlations between externally visible physical injuries and mortality were reported for *Chionoecetes* spp. (Rosenkranz, 2002) and red king crabs (Zhou and Shirley, 1995). However, internal injuries and bleeding that result in mortality can occur without apparent external injuries. Given that limitation, Stevens (1990) considered both external injuries and an index of spontaneous activity, termed vitality, as possible correlates with delayed mortality in red king crabs and Tanner crabs caught incidentally in Bering Sea trawls. The crabs were assessed and held for 48 hours in shipboard tanks to observe mortality. Logistic models showed that the vitality index was useful in predicting delayed mortality, and injury information did not significantly improve model power. Subsequently, others proposed that impaired righting behavior provides a sensitive indicator of freeze-related stress in snow crabs (Warrenchuk and Shirley, 2002), and might be used to predict mortality (van Tamelen, 2005). However, complex behaviors are difficult to quantify at sea because they require space and controlled conditions, and often do not yield graduated results. As an alternative to assessments determined by complex behavioral patterns, Davis and Ottmar (2006) recently discovered that easily acquired observations on a suite of simple reflex actions can provide excellent predictions of mortality in fishes related to both physical (i.e., wounding) and physiological (e.g., thermal stress, air exposure) injury.

We hypothesized that an assessment of reflex actions in crabs would directly reflect their condition and provide a good predictor for mortality, independent of external injury. The goals of this study were to identify reflexes for potential use in assessing crab condition, and then test their ability to predict mortality of *Chionoecetes bairdi* and *C. opilio* captured in Bering Sea trawls. Crabs were tested for reflex actions and injuries, and monitored for mortality in shipboard tanks. These experiments yielded excellent predictions of mortality based on reflex actions.

Materials and methods

Identification and scoring of individual reflexes

Laboratory studies were conducted at the Kodiak Fisheries Research Laboratory (National Marine Fisheries Service, Alaska Fisheries Science Center) during April 2007, to identify reflex actions of *Chionoecetes* spp. that could be reliably used in evaluating their likely survival. Thirty-two individuals of *C. bairdi* were collected by divers from Kodiak nearshore waters between February and April 2007. Males (n=24) ranged from 79 to 128 mm carapace width (CW). Females (n=8) ranged from 88 to 98 mm CW. The crabs were transported to the Kodiak Laboratory in buckets and placed in large fiberglass tanks (1.8 m diam., 1 m deep) where they were held with flowing seawater. Temperature ranged 1.5° to 4.6°C during the holding period. The crabs were fed twice each week with a diet of frozen chopped fish and squid. At the time of testing all of the crabs were in new shell condition, having molted in the past few months, and they were active and feeding. The crabs were marked with vinyl spaghetti tags tied loosely around the basiischium of the second or third walking leg. During the preliminary experiments seawater temperature was 5.0°C in the laboratory, and air temperature ranged 16° to 18.6°C.

From prior experience with various crab species, we expected that *C. bairdi* would demonstrate stereotypic responses to being lifted and to manipulation of their appendages. The goal was to identify simple reflex actions that could be evaluated rapidly in the tester's hand (out of water), during shipboard operations, with a high degree of reliability. After one day of manipulations we identified six reflex actions that appeared to be reliable (Table 1). These were tested on every individual crab on two consecutive days and were scored as strong, weak, or as no response.

Field experiment

A field experiment was conducted in June and July 2007 to evaluate the feasibility of using reflex actions as predictors of delayed mortality in *Chionoecetes* spp. Trawling operations for this study were conducted on the Bering Sea shelf east of St. Paul Island in the Pribilof Islands of Alaska (57°12′ to 57°25′N, 169°30′ to

Table 1

Reflexes identified as useful for assessing stress in *Chionoecetes* spp. The test was the manipulation required to elicit a stereotypic response. Characteristic strong and weak responses are described. When no motion was detected in response to repeated testing a "No response" score was recorded.

Reflex	Test	Strong response	Weak response
Leg flare	Lift crab by the carapace, dorsum up.	All legs spread wide and high, near horizontal orientation.	Legs droop below horizontal.
Leg retraction	While held as above, draw the forward-most walking legs in the anterior direction.	Legs respond with a strong retraction in the posterior direction.	Leg retraction is diminished. Low resistance to legs pulled forward.
Chela closure	Observe for motion or hold the chelae in the fingers.	Chelae open and close rapidly without manipulation. Manipulation results in immediate strong closure.	Chelae close slowly and weakly upon manipulation, or with a delayed response. Low resistance to manual opening of the chelae.
Eye retraction	Touch the eye stalk with a blunt probe, or lift the eye stalk from its retracted position.	Eye stalk retracts strongly in the lateral direction below the carapace hood.	Eye stalk retracts weakly or demonstrates low resistance to lifting.
Mouth closure	If closed, attempt to open (extend) the 3rd maxillipeds with a sharp dissecting probe. If open, draw the maxillipeds downward.	3rd maxillipeds retract quickly and strongly to cover the smaller mouth parts.	The maxillipeds droop open or move in an agitated manner, but do not close tightly.
Kick	With the crab in ventrum-up position, use a sharp dissecting probe to lift the abdominal flap away from the body.	Immediate, strong agitation of the legs and chelipeds. Males respond more strongly than females. Testing with the latter often requires greater extension of the entire abdominal flap.	Response is diminished or slow. Motion is observed in only the hind-most legs.

169°45′W) in depths ranging from 35 to 75 m. Crabs were collected from 22 locations where the bottom was relatively homogenous muddy sand. Temperature on the bottom and near surface ranged from −0.3° to 1.7°C and from 3.5° to 4.9°C, respectively.

An important objective of the trawling operation was to acquire crabs in various states of stress and injury for assessment and monitoring. Also, we wanted to test crabs that had experienced the suite of stressors typical of those produced during encounters with fishing gear used in the Bering Sea bottom-trawl fishery. Therefore, the crabs were collected in various locations around a commercial trawl by means of recapture nets (see Rose, 1999). The main trawl was a two-seam Alfredo bottom trawl (with headrope and footrope lengths of 36 and 54.6 m, respectively) similar to that used from many vessels in the Bering Sea. The center section of the footrope was composed of 46-cm diameter rounded cones separated by approximately 70-cm long sections of 20-cm diameter disks. The forward 14.2 m of the footrope on each wing of the trawl was made of 20-cm disks strung over a 19-mm long link chain. These sections were not directly attached to the netting panel above them, and thus formed so-called "flying wings." Extending forward from the trawl, 27.4-m bridles, made of bare cable, were attached to the upper wings and the same lengths of cable, covered with 9-cm diameter rubber disks, were attached to the lower wings. Ahead of these were 88 m of 4.8-cm diameter combination rope and 27.4 m of bare cable, leading to the trawl doors. The main trawl was towed with an open codend.

The recapture nets were small 2-seam trawls with longer headropes than footropes (14.3 m and 12.0 m, respectively). The long headrope maximized escape of fish, and the small diameter (5-cm) footropes were used to enhance crab capture. These nets were fished directly behind the main net sweeps, wings, or footrope. In some cases, two recapture nets were fished simultaneously at different locations. As a control for damage in the recapture nets, the nets were also fished ahead of the main trawl, capturing crabs with no previous damage. Tows were short (15 minutes) so that the stress or damage to crabs was created by the main trawl gear, and less by packing within the net or handling. However, evaluation of gear impact was not the primary goal of this cruise.

Once a recapture net was hauled on deck, the volume of the catch (normally comprising *Gadus macrocephalus*

Table 2

Composition of *Chionoecetes bairdi* (Tanner crab) and *C. opilio* (snow crab) populations tested and monitored in the field experiment. Values are means and ranges (in parentheses) for the nonrandom experimental population and do not represent the overall results of the fishing operations. Shell condition is a relative index of molt stage, ranging from molting or recently molted crabs with no hardening of the shell (0) to old and heavily encrusted shells (5). Reflex impairment index A is reported, representing the number of reflex actions that were entirely lost; this index can range from 0 to 6. Injury scores can range from 0 to 5, representing crabs with no visible exoskeletal damage to those with badly broken carapaces and limbs.

Species and gender	*n*	Carapace width (mm)	Shell condition	Reflex impairment	Injury scores	Mortality (%)
C. bairdi	250					29.2
Female	89	79 (58–97)	2.6 (2–4)	0.94 (0–6)	0.85 (0–5)	34.8
Male	161	98 (67–140)	2.7 (1–4)	0.73 (0–6)	0.66 (0–5)	26.1
C. opilio	399					24.3
Female	74	63 (52–81)	2.7 (2–4)	1.40 (0–6)	0.62 (0–5)	23.0
Male	325	83 (49–133)	2.6 (0–4)	1.42 (0–6)	0.72 (0–5)	24.6

[Pacific cod], *Atheresthes stomias* [arrowtooth flounder], and other unidentified flatfishes, seastars, and crabs) was estimated and *C. bairdi*, *C. opilio*, and *P. camtschaticus* were separated by species and gender into baskets. Air temperature during the crab handling process ranged 5° to 10°C. Sorting normally took <15 minutes and the sorting baskets were placed in fish totes with flowing seawater if the sorting and subsequent handling exceeded that time limit. The crabs were then tested individually for loss or weakness according to the six selected reflexes (loss=0, weak=1, strong=2) (Table 1), and notes were made on autotomy and obvious injuries such as broken legs, cracked carapace, or torn abdomen. Later, injuries were scaled from 0 to 5, where 0=no injuries, 1=newly autotomized legs, 2=broken legs, chelae, or mouthparts, 3=minor carapace or abdomen damage, 4=major carapace or abdomen damage, and 5=major damage to multiple parts (carapace, leg, and other parts). Crab gender, shell condition, and CW were recorded during the assessment period. Briefly, shell condition in *Chionoecetes* spp. was scored from 0 to 5, where 0 represented molting or recently molted crabs with no hardening of the shell whatsoever, crabs with shell condition 1 represented a soft flexible shell, condition 2 represented full hardness, and scores 3 to 5 showed increasing stages of discoloration and encrustation with shell age. Following assessment the crabs were either discarded overboard or tagged and held for monitoring mortality. Initially, all crabs were marked and held. However, it soon became apparent that a large proportion had relatively low reflex impairment. Thus, as the cruise progressed, emphasis was shifted to gear configurations producing greater damage to crabs, and we chose individuals with observable reflex impairment or injury for holding. As a result, the impairment, injury, and mortality rates reported in Table 2 do not represent the fishing conditions, only the crabs included in this analysis.

Crabs held for monitoring were marked with uniquely numbered vinyl spaghetti tags tied securely but loosely around the basi-ischium of the third leg, or fourth leg (if the third leg had been autotomized). Tagged crabs were immediately moved to one of 12 large fish totes (98×110×85 cm deep; ~900 liters) secured on the ship's trawl deck. Each tote was supplied with a constant flow of seawater (≥20 L/minute). Water temperature during 8–11 day holding periods ranged from 2.7° to 5.9°C, and oxygen (monitored morning and evening in every tote) never fell below 100% saturation. Mortality was assessed and dead crabs were removed each afternoon for the first five days of holding, then every other day until the end of the experiment. On the last day of holding all of the remaining crabs were re-assessed for reflex scores.

Reflex impairment indices and statistical procedures

Scores for reflex actions were combined into impairment indices and used in the analysis. Composites provided robust indices of overall condition for the animal and provided the advantage of reducing the weight of any one reflex (Davis, 2007). Analyses described below were conducted with two different impairment indices. Reflex impairment index A was calculated as the total number of zero scores for individual reflex actions (i.e., lost reflexes), and reflex impairment index B was the total number of scores that were either 0 or 1 (i.e., lost or weak reflexes). Both index scores ranged from 0 to 6.

Before analysis of the relationships between reflex impairment, injury, and mortality, we wanted to be certain that the holding period was sufficiently long to provide an accurate estimate for mortality. The time course for mortality was evaluated as a simple cumulative curve of deaths for *C. bairdi* and *C. opilio* shown as a function of time in days. We used simple linear regression to explore relationships among mortality-related variables, injury scores, and reflex impairment.

Logistic regression was used to model mortality, by using potential predictors and mediators including reflex impairment, injury score, gender, size, and shell

condition. Models were fitted by the method of maximum likelihood for binary data (i.e., dead or alive) with the regression module of Systat 12 (SYSTAT Software, Inc., San Jose, CA) (Peduzzi et al., 1980). A backward stepwise approach was used to determine the most parsimonious model for mortality, with an alpha value of 0.15 to remove a variable from the full model. This model for mortality was described by

$$Log_e(p/(1-p)) = \alpha + \beta' x,$$

Where p = proportion of $y = 1$;
y = 1 if crab was determined to be dead, and 0 if alive;
α = intercept;
β' = model coefficients; and
x = the model matrix of explanatory variables.

The maximum likelihood estimates of mortality (ρ) were calculated as

$$\rho = e^{(\alpha+\beta' x)}/1+e^{(\alpha+\beta' x)}.$$

Initially, the data for each species were split randomly into equal halves, one representing a learning set and the other a test set. The most parsimonious logistic model was developed with the learning set and validated with the test set. After cross-validation, a final model was fitted to the entire data set. Finally, the logistic model for each species was used to develop a surface plot showing the probability of mortality based upon fixed values for the key observations of crab condition.

Results

Laboratory results

The six reflex actions identified for testing with *C. bairdi* (Table 1) were highly reliable and consistent among individuals. Strong responses in leg flare, leg retraction, eye retraction, and mouth close were observed with every individual every time they were tested. Weak chela closure was observed just once, and the kick response to lifting the abdominal flap was weak in eight instances (seven females) and missing entirely in one test (also female). The weak responses generally occurred in the same individuals in duplicate trials.

Field evaluation of reflex impairment

Crabs collected in the recapture nets demonstrated a wide range of size, shell condition, reflex impairment, and injury level (Table 2), and reflex actions were lost at different frequencies. When just one reflex was absent, kick and leg retraction were the reflexes lost most frequently (Table 3). Among the crabs where one to five reflexes were lost, leg retraction, kick, and leg flare were most often lost, and eye retraction and mouth closure were absent least frequently.

Table 3

Percentages of reflex actions lost in *Chionoecetes bairdi* (Tanner crab) and *C. opilio* (snow crab). When just one reflex was absent in a crab. it was considered the 1st reflex lost. The right column represents the percentage of specific reflexes that were lost among all of the crabs where between one and five reflexes were lost.

Reflex	1st reflex lost	% of total losses
C. bairdi	(*n*=18)	(*n*=147)
Kick	44.4	26.5
Leg retraction	44.4	32.0
Leg flare	5.6	19.0
Chelae closure	5.6	14.3
Eye retraction	0	4.1
Mouth closure	0	4.1
C. opilio	(*n*=35)	(*n*=210)
Kick	51.4	23.3
Leg retraction	31.4	30.0
Leg flare	14.3	24.8
Chelae closure	0	13.8
Eye retraction	2.9	4.8
Mouth closure	0	3.3

Mortality increased with increasing reflex impairment, regardless of the specific index used (Fig. 1). However, mortality at a given impairment value was consistently higher with index A than with index B, and the differences were greatest between index values of 1 and 5. Hence, we judged index A, from the count of completely lost reflexes (scores=0), to provide a more sensitive predictor of mortality than the index based on reflexes scored as lost or weak. A lost reflex was more definitive and less ambiguous than a weak reflex; therefore, reflex impairment index A was used for all subsequent analyses.

Appropriateness of experimental holding

Adequacy of the field experiment depended upon two important experimental requirements. First, holding needed to be sufficiently long to allow detection of delayed mortality in crabs. Second, the holding methods themselves did not induce mortality.

Most of the mortality in the two species occurred within the first few days of collection (Fig. 2). For both species, all the crabs with reflex impairment equal to 6 died within the first hour after reflex assessment. Among the balance of the individuals with less impairment, 80% of the mortality occurred within 3 days for *C. bairdi* and 2 days for *C. opilio*. For both species, >95% died by day 7. Not surprisingly, time to mortality decreased with increasing reflex impairment (Fig. 3). Some crabs with low reflex impairment died in holding, but these crabs tended to have substantial physical injuries. Time to mortality averaged ~3 days for these individuals, and a high variability in time to mortal-

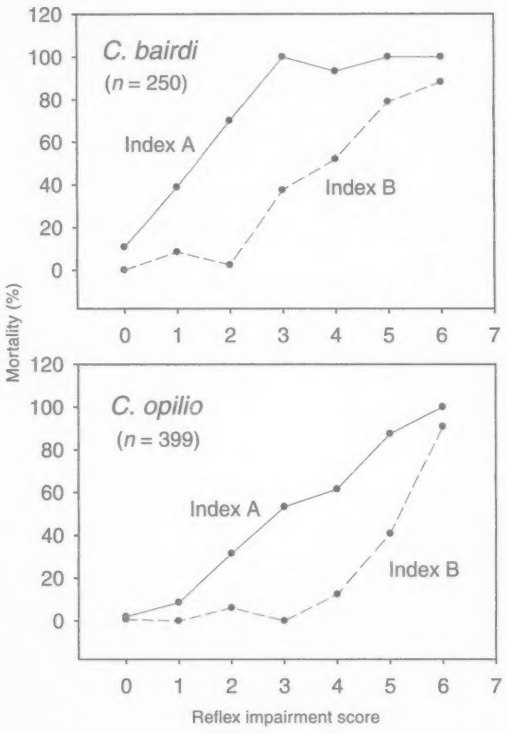

Figure 1

Percent mortality of *Chionoecetes bairdi* (Tanner crab) and *C. opilio* (snow crab) shown as a function of reflex impairment indices A and B. Index A was calculated as the sum of completely lost reflexes (score=0) and index B was calculated as the sum of reflexes either lost or weak (scores=0 or 1).

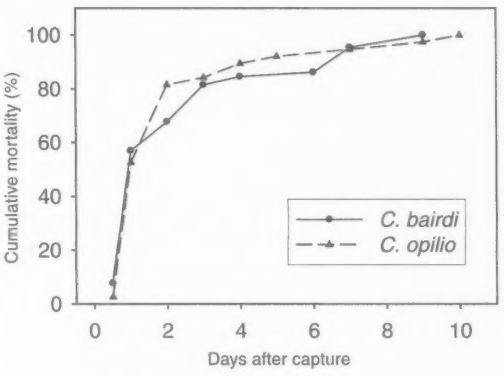

Figure 2

Percent cumulative mortality of *Chionoecetes bairdi* (Tanner crab) and *C. opilio* (snow crab) over time during holding at sea. Total numbers of *C. bairdi* and *C. opilio* dying over the course of the experiment were 73 and 96, respectively.

ity was observed for *C. opilio*. Crabs with high reflex impairment (4 to 6) usually died within 1 to 2 days and there was little variation in this period of mortality. From these results, we concluded that the holding period for the experiment (11 days) was sufficiently long to test the relationship between reflex impairment and mortality.

Re-assessment of reflex impairment in surviving crabs at the end of the experiment revealed that condition of the vast majority did not deteriorate during the holding period at sea. Only two of the surviving *C. bairdi* (n=176) had increased reflex impairment. One of these had multiple new leg autotomies and the other had multiple weak responses upon capture. Improvement in the reflex impairment index by one or two points occurred in 7.4% of the crabs, whereas the balance showed no changes. Only 8.5% of the survivors had reflex impairments >0 by the end of the study. Among the 295 *C. opilio* survivors, none had higher or lower reflex impairment, although most of the survivors (98%) had no impairment initially. These results indicate that the deck tanks provided a suitable means of holding to assess delayed mortality in the subject species.

Mortality and its relationship to reflex impairment

Logistic regression analysis revealed that mortality in *C. bairdi* was associated primarily with reflex impairment index and injury score (Table 4). Despite a wide range of crab size and shell condition (Table 2), neither these variables nor crab gender was a significant variable in the regression model. In the most parsimonious model, containing a constant, reflex impairment and injury score correctly predicted 82.7% of the mortality and survival (Table 4), whereas the full model (with all variables) predicted 82.8% correctly. Cross-validation showed that the model was robust; i.e., the random learning set produced a model that correctly predicted 86.7% of the test set for *C. bairdi*. When the injury score was removed from the regression model, 79.5% of the observed outcomes were predicted correctly, demonstrating the value of the assessed reflexes. A similar analysis for *C. opilio* revealed reflex impairment to be the only significant predictor of mortality (Table 5). The most parsimonious model, containing a constant and the reflex impairment index, correctly predicted 91.0% of the mortality and survival—a percentage almost identical to that of the full model (82.8%). As with *C. bairdi*, cross-validation for *C. opilio* with a learning set resulted in 94.0% correct predictions for the test set. Consequently, models for both species were robust for the field study.

Surface plots for probability of mortality with the logistic models for each species (Fig. 4) showed the strong relationships between reflex impairment and mortality. For *C. bairdi*, mortality increased rapidly

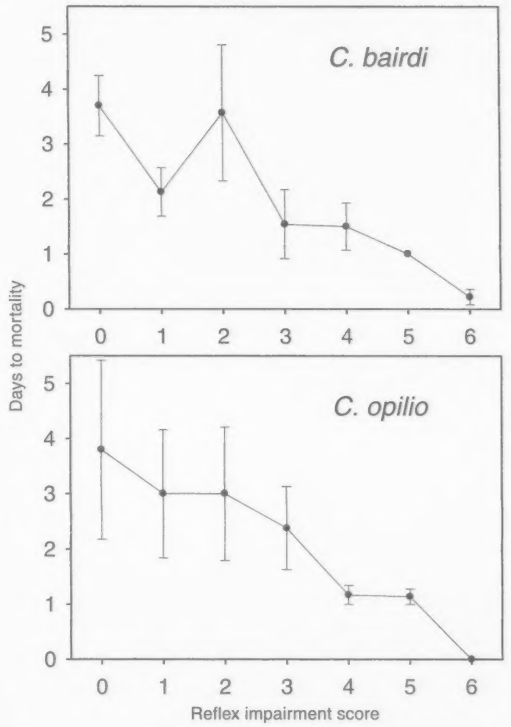

Figure 3

Time to mortality (days) observed for *Chionoecetes bairdi* (Tanner crab) and *C. opilio* (snow crab) shown as a function of reflex impairment (index A). Values are mean, ±standard error (in parentheses). Index A was calculated as the sum of completely lost reflexes (score=0) and index B was calculated as the sum of reflexes either lost or weak (scores=0 or 1).

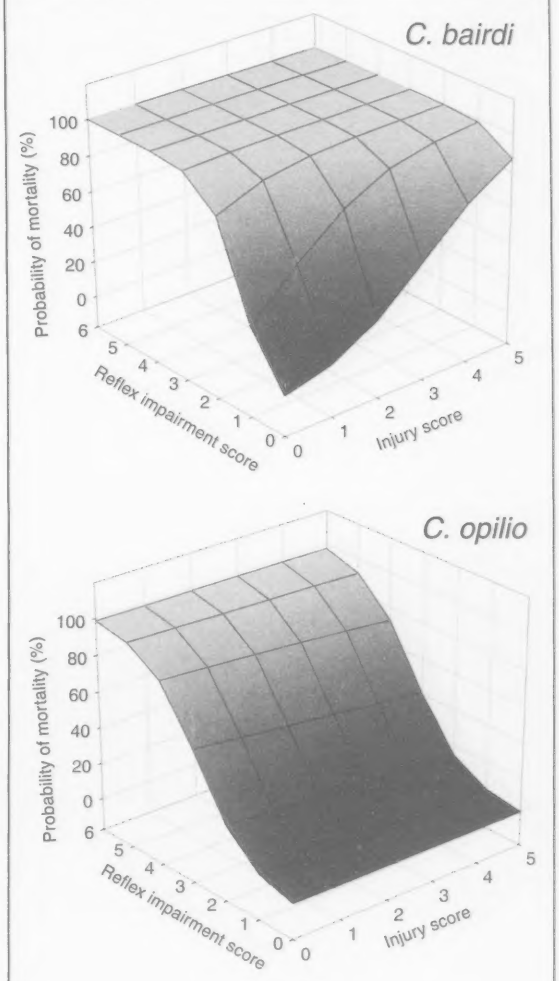

Figure 4

Surface plots, resulting from logistic regression, showing the probabilities of mortality for *Chionoecetes bairdi* (Tanner crab) and *C. opilio* (snow crab) with varied levels of reflex impairment (index A) and injury. Index A was calculated as the sum of completely lost reflexes (score=0) and index B was calculated as the sum of reflexes either lost or weak (scores=0 or 1).

with increasing impairment, reaching an asymptote at an index equal to 3. Mortality also increased with injury score, but the effect was smaller and more gradual. Probability of mortality in *C. opilio* was determined primarily by reflex impairment (with no obvious or significant effect of injury score). Reflex impairment and injury scores were correlated in both *C. bairdi* (R=0.514, $F_{1,248}$=89.024, P<0.001) and *C. opilio* (R=0.725, $F_{1,397}$=440.504, P<0.001).

The number of crabs representing each reflex impairment index (0 to 6) was not uniform, and we wanted to be certain that these unequal numbers did not bias the resulting relationship between mortality and reflex impairment. For example, among the 399 *C. opilio* tested, reflex impairments equal to 0, 1, and 6 were over-represented. Therefore, we randomly drew up to 20 individuals for each impairment level, reducing the number of crabs entering the model to 115. The reduced database resulted in a relationship between mortality and reflex impairment that was identical to that incorporating the larger data set. A similar analysis for *C. bairdi* produced a similar result, showing that the uneven distribution of data did not bias the experimental outcome and that the logistic models shown in Figure 4 were robust.

Discussion

Behavior of animals reflects a host of internal and external conditions, and, in the context of fishing-related

Table 4

Results of logistic modeling for mortality in *Chionoecetes bairdi* (Tanner crab). A backward stepwise approach was used to determine the most parsimonious model for mortality, and an alpha value of 0.15 was required to remove a variable from the full model.

Parameter	Estimate	t-ratio	*P*-value
Full model			
constant	−2.346	−1.226	0.220
gender	−0.114	−0.383	0.701
shell condition	−0.274	−0.805	0.421
size	0.005	0.244	0.807
reflex impairment	1.316	5.364	<0.001
injury score	1.029	4.743	<0.001
Most parsimonious model			
Parameter			
constant	−2.710	−8.957	<0.001
reflex impairment	1.353	5.731	<0.001
injury score	1.044	4.767	<0.001
Prediction matrix for the most parsimonious model	**No. dead predicted**	**No. alive predicted**	**Actual total**
Dead	51.4	21.6	73
Alive	21.6	155.4	177
Total predicted	73	177	250
Correct (%)	70.4	87.8	
False (%)	29.6	12.2	
Total correct (%)			82.7

Table 5

Results of logistic modeling for mortality in *Chionoecetes opilio* (snow crab). A backward stepwise approach was used to determine the most parsimonious model for mortality, and an alpha value of 0.15 was required to remove a variable from the full model.

Parameter	Estimate	t-ratio	*P*-value
Full model			
constant	−2.716	−1.933	0.053
gender	0.333	0.815	0.415
shell condition	−0.089	−0.292	0.770
size	−0.016	−1.128	0.259
reflex impairment	1.416	8.240	<0.001
injury score	−0.102	−0.384	0.701
Most parsimonious model			
Parameter			
constant	−3.913	−10.529	<0.001
reflex impairment	1.349	9.622	<0.001
Prediction matrix for the most parsimonious model	**No. dead predicted**	**No. alive predicted**	**Actual total**
Dead	78	18	96
Alive	18	285	303
Total predicted	96	303	399
Correct (%)	81.2	94.1	
False (%)	18.8	5.9	
Total correct (%)			91.0

injury, provides a neurological integration of both physiological stress and physical wounding (Davis, 2002). In fact, a variety of behaviors such as feeding (and growth) (Carls and O'Clair, 1990, 1995; Zhou and Shirley, 1995), molting (O'Brien et al., 1986; Carls and O'Clair, 1990), and righting behavior (Carls and O'Clair, 1990, 1995; Zhou and Shirley 1995; Warrenchuk and Shirley, 2002) are useful for evaluating crab condition. However, all these complex behaviors require some form of holding in seawater tanks for observation. For example, preliminary experiments at the Kodiak Fisheries Research Laboratory confirmed that lack of righting behavior in *C. bairdi* can be a useful predictor of mortality caused by both internal and externally visible injuries, but the evaluation can take >5 minutes, the behavior is highly variable, and yields only a binary response (i.e., yes or no). The vitality index described by Stevens (1990) also has just two possible outcomes—alive or moribund—for live crabs. In contrast, Davis and Ottmar (2006) and Davis (2007) have shown that testing a suite of reflex actions yields a graduated response variable with excellent potential for mortality prediction in fishes. They called the resulting relationship a reflex action mortality predictor (RAMP). Our results show that a similar approach is possible for *Chionoecetes* spp.

We identified six reflexes in *Chionoecetes* spp. that are stereotypic, repeatable, and easy to assess. These reflexes, associated with simple movements of the primary limbs, mouth parts, and eye stalks, represent the most fundamental and involuntary responses of the crabs, and they can be rapidly assessed in hand (i.e., without holding a crab in water). The most sensitive indicators of trawl-related stresses observed in this study were kick and leg retraction. These reflexes were generally lost first, followed by leg flare and chela closure, and reflexes associated with the eyes and mouth were least sensitive. The latter two reflexes probably require the lowest energy expenditures and movements of the mouth parts play an important role in ventilating the gills. Mouth movements were maintained in crabs near death and loss of motion could be used as the final determination of death (Stevens, 1990; this study). The order of reflex loss might shift somewhat under different fishing conditions or specific types of injury; however, a reflex impairment index, calculated by summing reflex actions without weighting individual reflexes (as in this study), has the advantage of representing condition over a wide range of stressor types in fishes (Davis and Ottmar, 2006; Davis, 2007). The same representation over a wide range of stressors is likely for crabs.

The most important finding of this study was that mortality in *Chionoecetes* spp. was closely correlated with reflex impairment. Although others have evaluated injuries to the exoskeleton in an attempt to predict mortality (Stevens, 1990), a thorough assessment of exoskeletal injuries is time consuming and minor and potentially fatal injuries, such as finely cracked carapaces, are easily missed. On the other hand, internal injuries and bleeding that can occur without external injury can contribute significantly to delayed mortality (Grant, 2003). Rose (1999) reported exoskeletal injury rates for red king crabs captured or struck with different types of trawl footropes, but it is now clear that these injury rates may or may not correlate well with mortality. Injury and reflex impairment were correlated in the present study, but our more general assessment of crab condition acquired through testing reflex actions provided a better predictor of mortality and an obvious improvement over tedious and subjective evaluations of shell damage. As observed with fishes (Davis, 2005, 2007), reflex impairment appears to integrate the effects of different kinds of stress and injury that can occur in gear encounters and in routine handling and discard practices.

Another key finding of this study was that the reflex impairment index provides a relatively universal indicator of condition and likely mortality for *Chionoecetes* spp. For example, shell condition can affect mortality rates in crabs handled in fishery operations and returned to sea (Kruse et al., 1994). However, our logistic analyses showed that reflex impairment indices provided the best predictors of mortality in both *C. bairdi* and *C. opilio*, regardless of shell condition and crab gender and size. Similarly, reflex impairment indices have provided excellent predictions of mortality for several different fish species over a wide range of sizes, and a good integration of cumulative stress (Davis and Ottmar, 2006; Davis, 2007).

Reflex action mortality predictors have also proven to be robust over a wide range of different stressor types, including tow time in a trawl, air exposure, and temperature shock, at least for fishes. The present study emerged from an interest in evaluating impacts of trawling operations on crab mortality rates and we believe that the response curves provided for *C. bairdi* and *C. opilio* should be robust for the kinds of injuries sustained under typical trawl operations in Alaska. The results, however, should be regarded primarily as a first proof of principle for crab species and the best possible RAMP models will depend upon additional fishing experiments conducted over a broader range of stressor types and fishing conditions. For example, other variables, such as freezing temperatures and windchill stress (Carls and O'Clair, 1995; Warrenchuk and Shirley, 2002) encountered in pot fishing warrant experimental investigation and may necessitate modifications to the mortality prediction models.

Once a RAMP curve is well developed for a species it should be widely applicable in fishing experiments designed to improve fishing practices and reduce discard mortality. Instead of the traditional approach, where fishing and handling variables are evaluated directly through multiday holding, either in deck boxes (Stevens, 1990) or in sea cages (Grant, 2003), crabs can be evaluated immediately in the field, without holding, and probabilities of mortality can be estimated with the RAMP model. For example, we have been interested in mortality of crabs that encounter trawls, but are not captured. In the future, we should be able to use recapture nets in front of and behind different trawl components (see

Rose, 1999), especially footropes, bridles, and sweeps, to quickly evaluate mortality for *Chionoecetes* spp. with the use of RAMP estimators. Neither assessments of exoskeletal injuries nor holding in cages or deck boxes will be required. Similarly, the sublethal and lethal effects of fishing conditions such as bottom type, water and air temperature, net packing, and time in air could be evaluated with a RAMP approach. Effects of soak times for pots and handling procedures could be assessed with the same tool. Also, with an increasing interest in the live market for crabs, the RAMP approach may be useful in perfecting shipping and holding procedures for the best possible live products. The largest direct benefit of RAMP approach will be to increase the scope and replication of experiments on gear and handling. More broadly, discard-related mortality is not constant, and we believe that this new approach will facilitate substantial improvements in fishery models that incorporate mortality for crabs, lobsters, and other crustaceans that are routinely discarded at sea.

Acknowledgments

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Fishes associated with pelagic *Sargassum* and open water lacking *Sargassum* in the Gulf Stream off North Carolina

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Abstract—The community structure of fishes associated with pelagic *Sargassum* spp. and open water lacking *Sargassum* was examined during summer and fall cruises, 1999–2003, in the Gulf Stream off North Carolina. Significantly more individual fishes (n= 18,799), representing at least 80 species, were collected from samples containing *Sargassum* habitat, compared to 60 species (n=2706 individuals) collected from open-water habitat. The majority (96%) of fishes collected in both habitats were juveniles, and planehead filefish (*Stephanolepis hispidus*) dominated both habitats. Regardless of sampling time (day or night), *Sargassum* habitat yielded significantly higher numbers of individuals and species compared with open-water collections. Overall, fishes collected by neuston net tows from *Sargassum* habitat were significantly larger in length than fishes collected from open-water habitat with neuston nets. A significant positive, linear relationship existed between numbers of fishes and the quantity of *Sargassum* collected by neuston net. Underwater video recordings indicated a layered structure of fishes among and below the algae and that smaller fishes were more closely associated with the algae than larger fishes. Observations of schooling behaviors of filefishes (Monacanthidae), dolphinfish (*Coryphaena hippurus*), and jacks (Carangidae), and fish-jellyfish associations were also recorded with an underwater video camera. Our data indicate that *Sargassum* provides a substantial nursery habitat for many juvenile fishes off the U.S. southeast coast.

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In the western North Atlantic Ocean, pelagic brown algae of the genus *Sargassum* form a dynamic, floating habitat that supports a diverse assemblage of fishes, invertebrates, sea turtles, pelagic birds, and marine mammals. The pelagic species *S. natans* and *S. fluitans* provide resources in an otherwise nutrient-poor environment and serve as a nursery area for many juvenile fishes (Wells and Rooker, 2004), some of which are commercially or recreationally important, or both (e.g., *Coryphaena hippurus* [dolphinfish], *Caranx* spp. [jacks], *Seriola* spp. [amberjacks]). *Sargassum* habitat appears to be particularly important for early survival of some fishes because the majority of fishes collected from *Sargassum* habitat in the Gulf of Mexico (Bortone et al., 1977; Wells and Rooker, 2004) and off the southeastern United States (Dooley, 1972; Moser et al., 1998) are juveniles.

The spatial distribution and quantity of *Sargassum* are highly variable. *Sargassum* distribution along the U.S. east coast depends on the Florida Current and the Gulf Stream, which entrain pelagic *Sargassum* from the Sargasso Sea and move it northward. Aggregations of *Sargassum* range from small, widely dispersed clumps to rafts and large weedlines that continue for many kilometers, and the great variability in the structure of this habitat is due to variations in Gulf Stream flow, storms, tidal currents, and wind-generated waves and currents. Although estimates of *Sargassum* biomass in the western North Atlantic have varied (Howard and Menzies, 1969; Butler and Stoner, 1984), the majority of pelagic *Sargassum* has persisted and reproduced vegetatively in the western North Atlantic Ocean for at least decades and probably for hundreds to thousands of years (Parr, 1939).

Sargassum habitat is extremely difficult to sample consistently and quantitatively, and no single method of sampling provides a complete view of the *Sargassum* community. Even though Moser et al. (1998) recommended using multiple sampling methods, especially visual methods, to survey this ecosystem, most *Sargassum* community studies from the Gulf of Mexico (Bortone et al., 1977; Wells and Rooker, 2004) and the western North Atlantic (Dooley, 1972; Moser et al., 1998) have been based on limited sampling methods. Kingsford (1995) and Dempster and Kingsford (2004) suggested that a weakness in previous studies was a lack of open-water, unvegetated control samples, and to date, in only one study (Moser et al., 1998) in the western North Atlantic have fishes associated with *Sargassum* habitat been compared to fishes in open-water habitat. Additionally, most samples of *Sargassum* were collected during daytime only (Dooley, 1972; Moser et al., 1998; Wells and Rooker, 2004) or sampling times were not specified (Bortone et al., 1977; Stoner and Greening, 1984). In some cases, explicit association of samples with *Sargassum* was unclear because samples were not collected within the algae (e.g., Settle, 1993).

The relationships between the quantity of *Sargassum* and species richness and abundance or biomass of fishes are highly variable. Dooley (1972) and Fedoryako (1980) found no correlation between numbers of fishes and quantity of *Sargassum*, but significant positive correlations between fish abundances and quantity of algae have been found in other studies (Moser et al., 1998; Wells and Rooker, 2004). Sampling methods may substantially influence these results. Nevertheless, it is clear that objects floating in the ocean, like *Sargassum*, attract and concentrate fauna (Kingsford, 1992).

Despite several surveys and the widespread occurrence of *Sargassum* habitat, the fishes associated with this habitat have not been extensively documented along the U.S. east coast. Our objective was to describe fish community structure (species composition, day versus night species composition, sizes, and *Sargassum* abundance and fish distribution relationships) within *Sargassum* and open-water habitats off North Carolina. In addition, behaviors of fishes within and below *Sargassum* habitat were documented to better describe the close associations of fishes with the habitat, the different types of habitat usage, and to provide a three dimensional view of the distribution of fishes within and beneath the *Sargassum*. Our approach was to use consistent methods (supplemented by further sampling) and extensive temporal (diel) and spatial sampling across ocean surface habitats with no *Sargassum* to those with high densities of *Sargassum* to examine the relative contribution of *Sargassum* to oceanic fish communities off the southeastern United States.

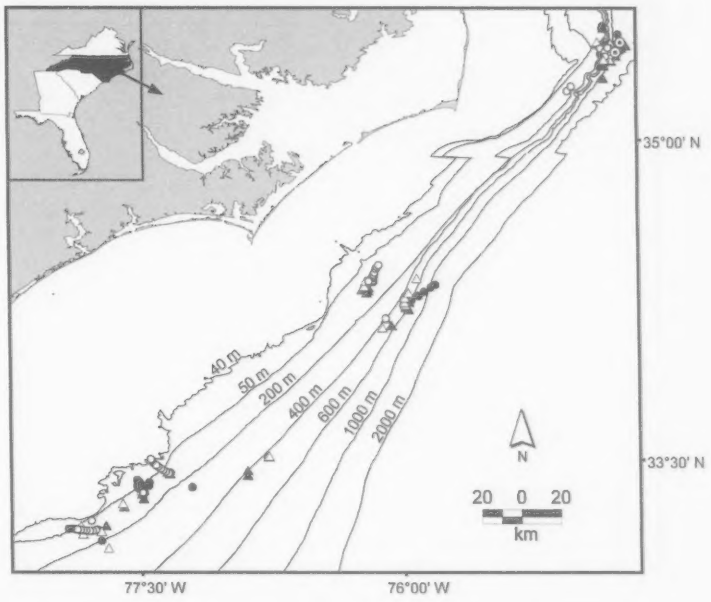

Figure 1

Surface sampling sites for fishes collected during summer and fall of 1999–2003 off North Carolina. Collections with neuston nets (triangles) and supplemental gears (circles) (i.e., nightlighting, dip net, hook-and line, longline) from *Sargassum* (closed symbols) and open-water (open symbols) habitats. The white circles with a black center off Cape Hatteras represent sites where underwater video recordings were taken.

Materials and methods

Sampling

Surface waters were sampled during 2–7 August 1999, 20–27 July 2000, 22–28 August 2001, 20–26 September 2001, 6–15 August 2002, and 17–26 August 2003, in the Gulf Stream off Cape Hatteras, Cape Lookout, and Cape Fear, North Carolina (Fig. 1). The primary sampling device, a 1.1×2.4-m neuston net (6.4-mm mesh body, 3.2-mm tailbag), was towed in the upper meter of the water column at slow speeds (<3.7 km/h) for 30 minutes in 1999 and for 15 minutes during 2000–03. Sampling was conducted throughout the 24-h period to compare daylight (0700–2000 h eastern daylight time [EDT]) and nighttime (–0700 h EDT) collections. The neuston net was towed in both open water without *Sargassum* and in waters with varying amounts of *Sargassum*. *Sargassum* is in constant motion in the Gulf Stream, and without aerial surveillance its distribution and density are unpredictable, especially at night. Because we were unable to consistently target a particular habitat, and thus balance sampling effort, the nets were pulled through unknown habitat and the sample was classified later depending on whether *Sargassum* was present in the sample or not (see *Data analysis*). In most cases it was also not possible to determine the proximity of one habitat to another. When *Sargassum* was abundant, the neuston net was towed directly through the clumps, mats, or weedlines, but on some occasions, *Sargassum* was collected opportunistically. Fishes were sorted from the algae in the neuston tow catches, and the *Sargassum* was weighed (wet weight) to the nearest 0.1 kg and discarded. Because neuston net tows in 1999 were of longer duration and catches were not handled consistently, the catch data from the 1999 neuston net tows were not analyzed statistically (see *Data analysis*).

Additional collection methods supplemented the use of neuston nets, especially when *Sargassum* was too dense for use of the neuston net. When conditions were favorable (low wind and waves), stations with nightlighting were sampled by allowing the vessel to drift with the current or maintain its slowest speed into the current. The deck lights of the vessel, plus two 500-W and one 1000-W spotlights, were used to illuminate surface waters around the stern and both sides of the vessel,

and fishes that swam near the vessel were collected by five or six crew members using small mesh (6.4-mm mesh) dip nets. Each 30-minute segment of time during the drift represented a station. During the nightlighting sampling, the presence or absence of *Sargassum* within the field of view was recorded, and if present, whether the *Sargassum* was collected in dip nets was recorded. Fishes were also opportunistically collected with dip nets during daylight when dense aggregations of *Sargassum* were encountered. Limited hook-and-line sampling occurred in both *Sargassum* and open-water habitats during the day and at night, and each sampling period (station) lasted from 15 to 160 minutes. One longline set was made in the Cape Hatteras study area. The line was about 366-m long and contained 104 baited hooks that fished within 1–2 m of the surface. The set was made at night, lasted for 501 min, and drifted for 30 km through open-water habitat.

In 1999, underwater video was recorded under a large *Sargassum* weedline at two stations off Cape Hatteras, North Carolina (Fig. 1). Snorkelers using a handheld color camcorder (SONY model DCR-TRV900, New York, NY) in a waterproof case swam at and just below (<3 m) the surface along the edge of and under the weedline. A total of 62 minutes of video footage was recorded during the two stations. Analyses of the underwater video footage included identification of species, documentation of behaviors, and placement of fishes within or below the weedline.

Specimens were preserved at sea in 10% formalin-seawater solution and later stored in 40% isopropanol. Larval fishes had been collected in previous *Sargassum* studies, and this fact implied an association with this habitat. However, because distributions of pelagic fish larvae are highly influenced by currents and they generally lack affinity for drift algae (Kingsford and Choat, 1985), their presence in *Sargassum* collections (Settle, 1993; Wells and Rooker, 2004) is probably coincidental. For this reason and because the neuston net mesh size was inappropriate for sampling larvae, larval fishes (classified according to Richards, 2006) were excluded from this study. Fishes were identified to the lowest possible taxon, counted, measured to the nearest mm for standard length (SL), and weighed (wet weight) to the nearest 0.1 g. Damage to some fishes precluded identification to species and SL measurements. When more than 500 individuals of the same species were collected in a tow, a subsample (approximately 10% of the catch) was measured for SL and wet weight.

Data analysis

Fish catches from neuston nets were analyzed statistically to assess differences in fish community structure between habitats, and diurnal differences in community structure. Neuston tows without *Sargassum* were designated as open-water (OW). Because clumps of algae as small as 0.005 kg could influence the distribution and abundance of fishes (Kingsford and Choat, 1985), samples were classified as *Sargassum* (S) if algae were collected, regardless of the quantity. The number of individuals and number of species collected from *Sargassum* and open-water habitats were log $(x+1)$ transformed before analysis to correct for heterogeneity of variance, to reduce the influence of abundant species, and to enhance the contribution of rare species. If the assumptions of homogeneity of variance and normality were not satisfied after data transformation, a nonparametric Mann-Whitney test was applied to determine whether there were differences in the number of individuals and species in *Sargassum* versus open-water habitat. A Kruskal-Wallis test was used to compare day and night fish catches from neuston nets within and across station types (i.e., S versus OW), and a Dunn's multiple comparison test was used to determine where significant differences occurred. The relationship of fish abundance and species richness to the quantity of *Sargassum* collected by neuston nets was evaluated with regression analysis. Length-frequency distributions for dominant species collected from *Sargassum* habitat were compared to the size structures of the same species collected from open-water habitat by using a Kolmogorov-Smirnov test.

Habitat type sampled (S versus OW) was also designated for the supplemental methods. If *Sargassum* was collected by dip net, the station was designated as S; otherwise it was OW. Likewise, if the hook-and-line gear was placed in *Sargassum* (S), the catch was designated as S; if the gear was placed in unvegetated habitat, the catch was designated as OW.

Results

Catch composition

For all methods and cruises combined, most fishes were collected in samples containing *Sargassum* habitat. A total of 18,799 fishes, representing 80 species from 28 families, were collected in 162 *Sargassum* samples, and a total of 2706 fishes, representing 60 species from 23 families, were collected in 80 open-water samples (Fig. 1; Table 1). Both *Sargassum* and open-water collections were dominated by the families Monacanthidae (75% of S, 45% of OW), Carangidae (13%, 21%), and Exocoetidae (6%, 19%). Individuals of nine species represented 93% of the total *Sargassum* catch (in decreasing order of abundance): *Stephanolepis hispidus* (planehead filefish), *Caranx crysos* (blue runner), *Cheilopogon melanurus* (Atlantic flyingfish), *Balistes capriscus* (gray triggerfish), *Seriola rivoliana* (almaco jack), *Parexocoetus brachypterus* (sailfin flyingfish), *Monacanthus ciliatus* (fringed filefish), *Decapterus punctatus* (round scad), and *Coryphaena hippurus* (dolphinfish). Individuals of 10 species represented 92% of the total open-water catch (in decreasing order of abundance): *S. hispidus*, *C. crysos*, *Clupea harengus* (Atlantic herring) (all from a single station), *C. melanurus*, *P. brachypterus*, *D. punctatus*, *Prognichthys occidentalis* (bluntnose flyingfish), *Oxyporhamphus micropterus* (smallwing flyingfish), *Istiophorus platypterus* (sailfish), and *C. hippurus*. For all methods

Table 1

Number (and standard length [in mm] ranges in parentheses) of fishes collected by method from *Sargassum* and open-water habitats during summer and fall of 1999–2003 off North Carolina. NN=neuston net, NL=nightlighting, DN=dip net, HL=hook-and-line, LL=longline, number of stations is in parentheses under gear type. † =total length in mm, N/A=length not available due to specimen damage, *=species not previously collected in association with *Sargassum*. Identification to species was not possible for some fishes because of damage. Species are listed in phylogenetic order.

	Sargassum					Open water				
Taxon	NN (95)	NL (42)	DN (18)	HL (7)	Total *n*	NN (39)	NL (33)	HL (7)	LL (1)	Total *n*
Carcharhinidae										
Carcharhinus falciformis				1 (890)	1					0
Ophichthidae										
*Ahlia egmontis**		4 (270–410)			4		1 (378)			1
Clupeidae										
Clupea harengus					0		216 (22–42)			216
Gonostomatidae										
Cyclothone sp.*	1 (N/A)				1					0
Sternoptychidae										
*Argyropelecus aculeatus**	1 (41)				1					0
Phosichthyidae										
Vinciguerria poweriae					0		1 (20)			1
Synodontidae										
Synodus synodus					0		1 (31)			1
Myctophidae										
*Diaphus dumerilii**	1 (61)				1					0
*Myctophum affine**	27 (13–27)				27					0
*Myctophum obtusirostre**	10 (13–53)				10					0
*Myctophum punctatum**	5 (39–51)				5					0
*Myctophum selenops**	1 (45)				1					0
Myctophum sp.	1 (18)				1					0
Antennariidae										
Histrio histrio	13 (9–45)	4 (7–36)	6 (12–46)		23					0
Mugilidae										
Mugil curema	1 (14)				1	4 (14–20)	4 (25–27)			8
Unidentified	2 (13–14)				2					0
Belonidae										
*Ablennes hians**	14 (33–374)	8 (90–393)			22	3 (40–126)	4 (351–466)			7
*Platybelone argalus**	2 (149–155)	3 (149–236)			5					0
Tylosurus acus	18 (53–336)	14 (63–481)			32		4 (202–285)			4
*Tylosurus crocodilus**		1 (201)			1					0
Tylosurus sp.	2 (44–57)				2					0
Exocoetidae										
*Cheilopogon cyanopterus**	6 (24–112)	3 (118–144)			9		8 (30–131)			8
*Cheilopogon exsiliens**	2 (31–115)	2 (112–122)			4		6 (20–108)			6
Cheilopogon furcatus	2 (19–30)	6 (35–120)			8		4 (35–112)			4

continued

Table 1 (continued)

Taxon	*Sargassum* NN (95)	NL (42)	DN (18)	HL (7)	Total *n*	Open water NN (39)	NL (33)	HL (7)	LL (1)	Total *n*
Exocoetidae (cont.)										
Cheilopogon melanurus	399 (13–205)	157 (21–253)	2 (17–32)		558	57 (12–56)	147 (28–257)			204
Cheilopogon sp.	6 (19–23)				6					0
*Cypselurus comatus**	1 (72)				1		1 (107)			1
Exocoetus obtusirostris		1 (137)			1		3 (18–71)			3
Hirundichthys affinis	13 (17–95)	18 (56–164)			31	6 (17–47)	16 (49–105)			22
*Oxyporhamphus micropterus**	2 (64–95)	83 (33–183)			85		43 (12–110)			43
Parexocoetus brachypterus	160 (8–72)	86 (15–113)			246	46 (8–42)	112 (13–128)			158
*Prognichthys occidentalis**	36 (11–74)	45 (17–147)			81	7 (11–35)	62 (15–165)			69
Hemiramphidae										
*Euleptorhamphus velox**	11 (45–216)	3 (180–318)			14	9 (44–75)	6 (56–388)			15
Hemiramphus balao	10 (37–90)	20 (32–198)			30		2 (95–104)			2
*Hemiramphus brasiliensis**	22 (33–84)	7 (45–114)			29	2 (37–52)	3 (58–111)		4 (236–242)	9
Hemiramphus sp.	33 (31–75)				33		5 (24–110)			5
*Hyporhamphus unifasciatus**	1 (30)				1					0
Holocentridae										
Holocentrus sp.					0		1 (62)			1
Syngnathidae										
*Bryx dunckeri**	4 (28–47)		4 (35–42)		8	1 (32)				1
Hippocampus erectus	12 (17–143)†				12	2 (25–67)†				2
*Hippocampus reidi**	2 (72)†				2					0
Hippocampus sp.	1 (24)†				1					0
Syngnathus louisianae	1 (121)				1					0
Syngnathus pelagicus	1 (117)		15 (78–158)		16	1 (110)				1
Fistularidae										
*Fistularia tabacaria**	1 (69)				1					0
Fistularia sp.					0	1 (138)				1
Acropomatidae										
*Synagrops bellus**	1 (45)				1					0
Echeneidae										
*Echeneis naucrates**				2 (630–680)	2			1 (665)		1
*Remora osteochir**	1 (26)				1					0
Coryphaenidae										
*Coryphaena equiselis**	2 (26–32)	18 (28–68)			20	1 (30)	6 (37–91)			7
Coryphaena hippurus	99 (26–262)	22 (32–178)		41 (275–1020)	162	7 (26–112)	7 (27–188)	23 (370–623)	1 (286)	38
Carangidae										
Alectis ciliaris					0	4 (12–15)				4

continued

Table 1 (continued)

Taxon	*Sargassum* NN (95)	NL (42)	DN (18)	HL (7)	Total *n*	Open water NN (39)	NL (33)	HL (7)	LL (1)	Total *n*
Carangidae (cont.)										
Caranx bartholomaei	3 (20–61)	1 (34)			4					0
Caranx crysos	1646 (11–82)	91 (12–65)	96 (11–61)		1833	271 (11–72)	73 (12–99)			344
*Caranx lugubris**		3 (21–29)			3					0
Caranx ruber	57 (16–89)	2 (31–68)			59					0
Caranx sp.	20 (11–21)	3 (15–21)	18 (11–22)		41	12 (11–18)	2 (11–17)			14
Decapterus punctatus	144 (10–44)	19 (10–32)	3 (24–35)		166	150 (10–44)	1 (18)			151
Decapterus sp.	11 (16–30)		1 (20)		12		1 (28)			1
Elagatis bipinnulata	18 (17–81)	5 (24–43)			23		1 (19)			1
Selar crumenophthalmus	4 (17–29)				4	4 (15–21)	1 (28)			5
*Selene setapinnis**			1 (19)		1	1 (13)				1
Selene vomer					0	3 (11–13)				3
Selene sp.					0	1 (14)				1
Seriola dumerili	7 (11–70)	3 (37–47)	1 (48)		11					0
Seriola fasciata	59 (12–107)	1 (26)	2 (29–32)		62	4 (23–30)				4
Seriola rivoliana	260 (12–95)	4 (16–57)	4 (20–36)		268	13 (21–47)	3 (14–34)			16
Seriola zonata	4 (23–37)				4	1 (16)				1
Seriola sp.	21 (12–66)	4 (13–71)	8 (11–22)		33	4 (11–16)	1 (12)	1 (N/A)		6
Trachinotus carolinus					0		2 (21–23)			2
Trachinotus falcatus					0	1 (14)				1
Lobotidae										
Lobotes surinamensis	11 (11–52)	2 (29–30)	1 (33)		14					0
Mullidae										
*Mulloidichthys martinicus**		1 (87)			1					0
Pseudupeneus maculatus		3 (37–43)			3		1 (26)			1
Kyphosidae										
Kyphosus incisor	2 (29–91)	4 (19–39)	1 (14)		7					0
Kyphosus sectatrix	30 (15–89)	2 (25–33)			32					0
Kyphosus sp.	1 (15)				1		2 (19–21)			2
Pomacentridae										
Abudefduf saxatilis	45 (16–29)	2 (18–19)	4 (16–20)		51					0
Sphyraenidae										
Sphyraena barracuda					0			1 (1219)		1
*Sphyraena borealis**	1 (31)				1					0
Gempylidae										
Gempylus serpens					0		3 (64–81)			3
Scombridae										
*Auxis thazard**		2 (62–86)			2					0
Katsuwonus pelamis					0			2 (438–485)		2
Scomber colias					0			27 (225–261)		27
Scomber scombrus					0				6 (243–268)	6

continued

Table 1 (continued)

Taxon	*Sargassum* NN (95)	NL (42)	DN (18)	HL (7)	Total *n*	Open water NN (39)	NL (33)	HL (7)	LL (1)	Total *n*
Istiophoridae										
*Istiophorus platypterus**	4 (22–41)	4 (32–118)			8	2 (17–24)	38 (20–204)			40
Makaira nigricans					0		1 (205)			1
Nomeidae										
Psenes cyanophrys	5 (35–52)				5					0
Balistidae										
Balistes capriscus	345 (10–74)	19 (12–113)	37 (11–63)		401	17 (10–35)	2 (19–25)			19
Canthidermis maculata	17 (11–24)		2 (14–22)		19					0
Canthidermis sufflamen	26 (11–18)		1 (45)		27	1 (13)				1
Xanthichthys ringens	1 (21)	1 (65)			2					0
Unidentified	2 (N/A)				2					0
Monacanthidae										
Aluterus heudelotii	69 (25–70)	1 (50)	4 (31–44)		74	1 (24)	2 (36–39)			3
Aluterus monoceros	34 (33–151)	6 (53–95)			40	1 (52)	1 (103)			2
Aluterus schoepfii	21 (18–69)				21	9 (16–38)	1 (46)			10
Aluterus scriptus	42 (20–113)	5 (36–122)			47		1 (32)			1
Aluterus sp.	3 (15–26)				3					0
Cantherhines macrocerus	6 (30–60)	1 (96)			7					0
Cantherhines pullus	14 (30–67)	1 (59)			15	2 (42–60)				2
Monacanthus ciliatus	176 (10–26)		6 (16–27)		182	7 (12–17)	1 (21)			8
Monacanthus tuckeri	14 (16–24)		4 (17–25)		18					0
Monacanthus sp.	1 (14)				1	1 (13)				1
Stephanolepis hispidus	12,420 (8–65)	288 (8–57)	1064 (8–68)		13,772	1161 (8–49)	16 (10–76)			1177
Stephanolepis setifer	1 (40)				1					0
Stephanolepis sp.					0	2 (10–11)				2
Tetraodontidae										
*Lagocephalus lagocephalus**		1 (127)			1					0
Diodontidae										
Chilomycterus sp.	1 (22)				1					0
Diodon holocanthus	6 (15–55)	3 (46–75)			9		1 (51)			1
Diodon hystrix	2 (37–219)	1 (85)			3		2 (160–212)			2
Total	16,482	988	1285	44	18,799	1820	820	55	11	2706

combined, 33 species were collected only in association with *Sargassum* habitat, and 13 species were unique to open-water habitat (Table 1).

There was a large discrepancy between *Sargassum* and open-water catches from the 2000–03 neuston net samples. A total of 14,123 fishes, representing 65 species, were collected in 91 neuston tows in *Sargassum* habitat. Thirteen open-water tows produced no catch, whereas 14 open-water tows yielded 1393 fishes, representing 27 species (Table 2). Dominant families collected by neuston net in both *Sargassum* and open-water habitats were Monacanthidae (83% of S, 79% of OW), Carangidae (9%, 13%), and Exocoetidae (4%, 6%). Individuals of eight species represented 95% of the total *Sargassum* catch with neuston nets (in decreasing order of abundance): *S. hispidus*, *C. crysos*, *C. melanurus*, *B. capriscus*, *M. ciliatus*, *S. rivoliana*, *P. brachypterus*, and *C. hippurus*. Individuals of four species represented 93%

Table 2

Number of fishes collected in neuston net tows from *Sargassum* and open-water habitat off North Carolina during 2000–03, separated by day and night. Number of samples is in parentheses. Species are listed in phylogenetic order.

	Sargassum		Open water			*Sargassum*		Open water	
Species	Day (47)	Night (44)	Day (19)	Night (8)	Species	Day (47)	Night (44)	Day (19)	Night (8)
Cyclothone sp.	1	0	0	0	*Caranx bartholomaei*	2	1	0	0
Argyropelecus aculeatus	1	0	0	0	*Caranx crysos*	342	468	31	43
Diaphus dumerilii	1	0	0	0	*Caranx ruber*	23	33	0	0
Myctophum affine	0	27	0	0	*Caranx* spp.	2	18	1	1
Myctophum obtusirostre	0	10	0	0	*Decapterus punctatus*	9	44	78	1
Myctophum punctatum	1	4	0	0	*Decapterus* spp.	4	7	0	0
Myctophum selenops	0	1	0	0	*Elagatis bipinnulata*	5	5	0	0
Myctophum sp.	0	1	0	0	*Selar crumenophthalmus*	2	0	0	0
Histrio histrio	7	2	0	0	*Selene setapinnis*	0	0	1	0
Mugilidae	0	2	0	0	*Seriola dumerili*	3	4	0	0
Ablennes hians	6	8	1	2	*Seriola fasciata*	30	21	4	0
Platybelone argalus	0	2	0	0	*Seriola rivoliana*	125	35	13	0
Tylosurus acus	1	2	0	0	*Seriola* spp.	12	9	3	1
Tylosurus spp.	0	2	0	0	*Seriola zonata*	0	4	1	0
Cheilopogon cyanopterus	0	6	0	0	*Lobotes surinamensis*	9	1	0	0
Cheilopogon exsiliens	0	2	0	0	*Kyphosus incisor*	0	2	0	0
Cheilopogon furcatus	0	2	0	0	*Kyphosus sectatrix*	11	1	0	0
Cheilopogon melanurus	31	365	5	49	*Kyphosus* sp.	1	0	0	0
Cheilopogon spp.	0	6	0	0	*Abudefduf saxatilis*	5	5	0	0
Cypselurus comatus	0	1	0	0	*Istiophorus platypterus*	1	3	1	1
Hirundichthys affinis	0	13	0	6	*Psenes cyanophrys*	0	5	0	0
Oxyporhamphus micropterus	0	2	0	0	*Balistes capriscus*	120	109	9	5
					Canthidermis maculata	9	1	0	0
Parexocoetus brachypterus	19	140	2	17	*Canthidermis sufflamen*	23	3	0	1
Prognichthys occidentalis	9	26	1	6	*Xanthichthys ringens*	1	0	0	0
Euleptorhamphus velox	1	10	0	2	Balistidae	0	2	0	0
Hemiramphus balao	3	7	0	0	*Aluterus heudelotii*	48	17	0	0
Hemiramphus brasiliensis	0	21	1	0	*Aluterus monoceros*	1	3	0	1
Hemiramphus spp.	2	31	0	0	*Aluterus schoepfii*	10	7	0	1
Hyporhamphus unifasciatus	0	1	0	0	*Aluterus scriptus*	31	8	0	0
					Aluterus sp.	0	1	0	0
Bryx dunckeri	2	1	1	0	*Cantherhines macrocerus*	2	3	0	0
Hippocampus erectus	3	8	0	1	*Cantherhines pullus*	3	9	2	0
Hippocampus reidi	1	1	0	0	*Monacanthus ciliatus*	75	97	1	6
Hippocampus sp.	1	0	0	0	*Monacanthus tuckeri*	9	4	0	0
Syngnathus louisianae	1	0	0	0	*Monacanthus* sp.	1	0	0	0
Syngnathus pelagicus	1	0	1	0	*Stephanolepis hispidus*	7840	3541	681	408
Fistularia sp.	0	0	1	0	*Stephanolepis setifer*	1	0	0	0
Synagrops bellus	0	1	0	0	*Chilomycterus* sp.	0	1	0	0
Coryphaena equiselis	2	0	0	1	*Diodon holocanthus*	1	5	0	0
Coryphaena hippurus	13	71	1	0	*Diodon hystrix*	1	1	0	0
					Total	8869	5254	840	553

of the total open-water catches with neuston nets (in decreasing order of abundance): *S. hispidus*, *D. punctatus*, *C. crysos*, and *C. melanurus*. There were significantly more individuals (Mann-Whitney test: df=117, $P<0.001$) and numbers of species (Mann-Whitney test: df=117, $P<0.001$) in *Sargassum* habitat compared with open-water habitat. The three most abundant species in neuston net collections containing *Sargassum* habitat also exhibited the highest frequencies of occurrence: *S. hispidus* (70% of samples), *C. crysos* (64%), and *C. melanurus* (46%), whereas in open-water habitat these species occurred in 41%, 19%, and 22% of samples, respectively. Forty of the total 65 species collected in 2000–03 neuston net tows in *Sargassum* were unique to this habitat, whereas only two (*Fistularia* sp., *Selene setapinnis*) of the total 27 species collected in open-water habitat were unique (Table 2).

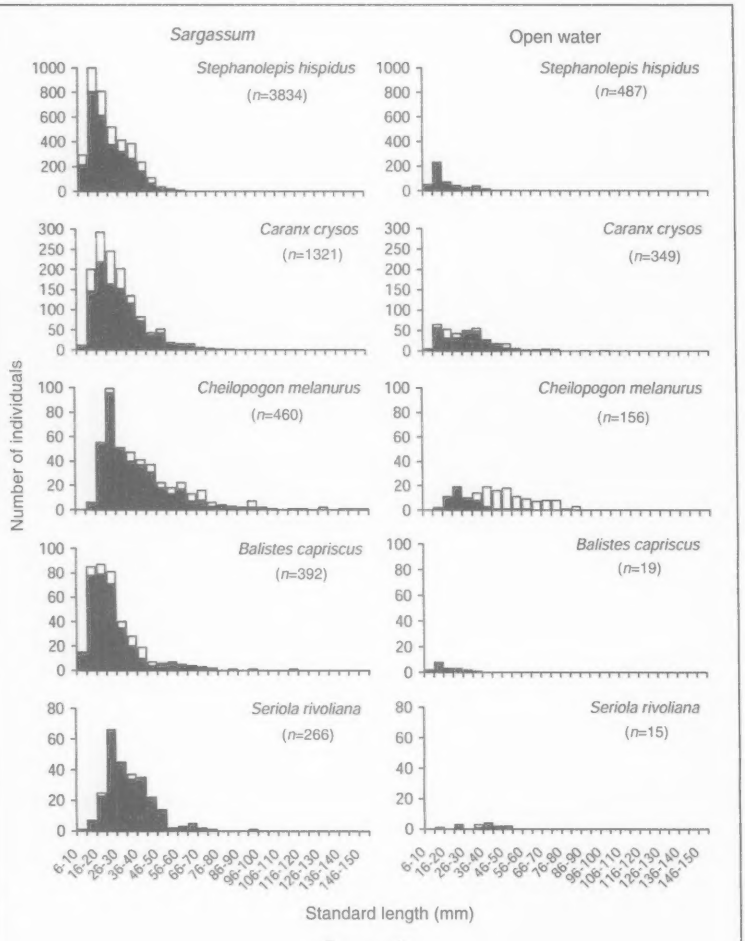

Figure 2

Length-frequency distributions of the nine most abundant fishes collected from *Sargassum* and open-water habitats during summer and fall of 1999–2003 off North Carolina. Neuston net collections = black bars and supplemental gears = white bars. Only juvenile specimens were graphed for *Cheilopogon melanurus* (Atlantic flyingfish) and *Coryphaena hippurus* (dolphinfish). Note differences in *y*-axis scale.

Day versus night catch composition

Regardless of sampling time (day or night), *Sargassum* habitat yielded significantly (Kruskal-Wallis test: df=3, $P<0.05$) higher numbers of individuals and species than open-water habitat. Daytime neuston net samples from *Sargassum* habitat ($n=47$) yielded 8869 fishes from 48 species, and nighttime neuston net samples from *Sargassum* habitat ($n=44$) yielded 5254 fishes from 56 species (Table 2); however, these differences were not statistically significant (Kruskal-Wallis test: df=3, $P=0.924$). Supplemental methods used in *Sargassum* habitat (dip nets, hook-and-line, 1999 neuston net) yielded different results; slightly more fishes (350 individuals) were collected at night than during the day. This finding was likely due to the slightly higher effort at night and the attraction of fish by the nightlighting.

As above, most fishes from neuston net samples in open-water habitat were collected during the day. Ten of the total 19 daytime neuston net tows in open-water habitat yielded 840 fishes, representing 20 species, and four of the total eight nighttime neuston net samples in open water yielded 553 fishes, representing 18 species (Table 2); however, these differences were not statistically significant (Kruskal-Wallis test: df=3, $P=0.843$). Supplemental methods used in open-water habitat (dip nets, hook-and-line, 1999 neuston net, long line), as above, produced more fishes at night (213 more individuals), probably for the same reasons.

Size distributions

Ninety-six percent of all fishes collected in surface waters during these summer and fall cruises were juveniles and most (88%) were ≤50 mm SL. The majority of *S. hispidus* (79% S, 87% OW), *C. crysos* (72%, 61%), *B. capriscus* (79%, 95%), *M. ciliatus* (100%, 100%), and *D. punctatus* (93%, 86%) collected in both *Sargassum* and open-water habitats were ≤30 mm SL (Fig. 2). *Cheilopogon melanurus* collected

in *Sargassum* habitat ranged from 13 to 253 mm SL, but the majority (84%) were juveniles <150 mm SL. *Cheilopogon melanurus* collected in open-water habitat ranged from 12 to 257 mm SL, and the majority (77%) were also juveniles <150 mm SL (Fig. 2). *Coryphaena hippurus* collected in *Sargassum* habitat ranged from 25 to 1020 mm SL, the majority (80%) of which were at the juvenile stage (<300 mm SL), and *C. hippurus* collected in open-water habitat ranged from 26 to 623 mm SL, 45% of which were juveniles (<300 mm SL) (Fig. 2).

Overall, fishes collected from neuston net tows containing *Sargassum* habitat (8–374 mm SL, mean=26 mm [±0.2 mm]) were significantly larger (Kolmogorov-Smirnov test: df=7464, $P<0.001$) than fishes collected from open-water habitat (8–138 mm SL, mean=23 mm [±0.4]) by the same method. Individuals collected in neuston net tows with *Sargassum* were significantly larger (Kolmogorov-Smirnov test, $P<0.05$) than individuals of the same species collected in neuston tows in open water for seven of the nine most abundant species (Table 3).

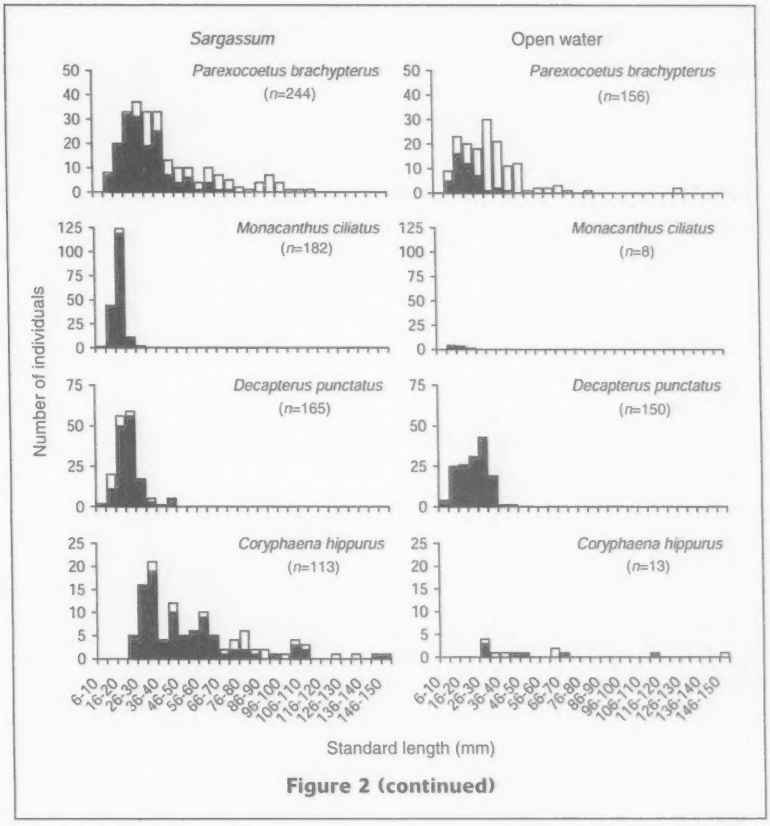

Figure 2 (continued)

Sargassum abundance and fish distribution

Despite variability, the quantity of *Sargassum* habitat was correlated with fish species richness and density. A significant positive linear relationship existed between the overall numbers of fishes and *Sargassum* wet weight from neuston net samples (Fig. 3A). For five (*S. hispidus, C. melanurus, B. capriscus, S. rivoliana, P. brachypterus*) of the eight most abundant *Sargassum*-associated fish species collected by neuston net during 2000–03, a significant positive relationship existed between numbers of individuals and *Sargassum* wet weight (Fig. 4).

Table 3

Mean standard length (SL) (±standard error [SE] in mm) of abundant fish species collected with neuston nets from *Sargassum* (S) and open-water (OW) habitats off North Carolina during 1999–2003. *n*=number of fishes, * = statistically significant (Kolmogorov-Smirnov test, $P<0.05$).

	Mean SL ±SE (mm)			
Species	S	*n*	OW	*n*
Stephanolepis hispidus	22* (±0.2)	2872	18 (±0.4)	470
Caranx crysos	28* (±0.4)	1007	27 (±0.7)	276
Cheilopogon melanurus	35* (±0.8)	392	27 (±1.2)	57
Balistes capriscus	23* (±0.7)	336	17 (±1.7)	17
Seriola rivoliana	32 (±0.7)	257	35 (±2.6)	12
Monacanthus ciliatus	17* (±0.2)	176	14 (±0.8)	7
Parexocoetus brachypterus	31* (±1.0)	158	22 (±1.0)	44
Decapturus punctatus	21 (±0.5)	143	23* (±0.6)	149
Coryphaena hippurus	40* (±2.6)	142	29 (±5.1)	21

Although a significant positive logarithmic relationship was observed between numbers of species and *Sargassum* wet weight (Fig. 3B), similar numbers of species were often collected regardless of *Sargassum* quantities. For example, the maximum number of species (n=19) collected in one neuston tow coincided with a relatively low quantity of *Sargassum* habitat (6.8 kg) (Fig. 3B).

Behavioral observations

Underwater video recordings clarified the close association of juvenile fishes to structure compared with open water. Many juvenile fishes rapidly explored and associated with any new substrata introduced near the *Sargassum* mats (e.g., snorklers, vessel). As in our other collections, the two most abundant families of fishes observed in the video recordings were Monacanthidae (mostly *S. hispidus*) and Carangidae (*Caranx* spp. and *Seriola* spp.). Fishes exhibited a size-related layering among and below the *Sargassum* (Fig. 5, A–C). Smaller juvenile fishes were usually very close to or within the *Sargassum* and were rarely observed more than one meter below the algae (Fig. 5A), whereas larger, more mobile juvenile fishes (e.g., carangids and kyphosids) were further below the *Sargassum* (Fig. 5B). Even deeper below the *Sargassum* (up to 3 m), larger predators (e.g., adult dolphinfish and jacks) were observed, usually in schools (Fig. 5C). When large predators swam below the *Sargassum*, smaller fishes moved upward into the algae (Fig. 5A).

Other behaviors were also observed from the underwater video recordings. Individuals and groups (7–10 individuals) of juvenile *Aluterus monoceros* (with a light to dark brown mottled pattern, Fig. 5D) were observed hovering just below the *Sargassum* with heads down at a 45° angle, tails near the surface (Fig. 5D). A school (about 65 individuals) of adult *A. monoceros* (silver body color) exhibited the same behavior under the hull of the vessel, which was adjacent to the weedline. On 15 occasions, small groups (2–15 individuals) of juvenile *S. hispidus* were observed pursuing and nipping at lobate ctenophores, *Mnemiopsis leidyi* (Fig. 5E). These interactions took place about 1.5 m below the *Sargassum*, and *S. hispidus* was the only species observed displaying this behavior. A distinct boundary was observed between open-water and *Sargassum* habitats; the open water adjacent to the edge of the weedline was unpopulated, whereas a high density of juvenile fishes were observed underneath and within the *Sargassum* (Fig. 5F).

Schools of adult dolphinfish (approximately 10–50 individuals) were observed swimming under the weedline on seven occasions. Most of these appeared to be females based on head shape and estimated sizes, but some may have been immature males. A female (295 mm SL), ripe with eggs, was collected from the vessel by hook-and-line during video recording operations. On five of the seven occasions, adult *Caranx bartholomaei* and *C. crysos* were mixed with the school of dolphinfish or swam closely behind them. On one occasion, a single large juvenile dolphinfish (approximately 300 mm SL) swam rapidly upward into the *Sargassum* with its mouth open, turned away at the weedline, and swam away; however, no small fishes were observed under the *Sargassum* in the vicinity of the strike, and it was not possible to determine the success of this apparent feeding attempt.

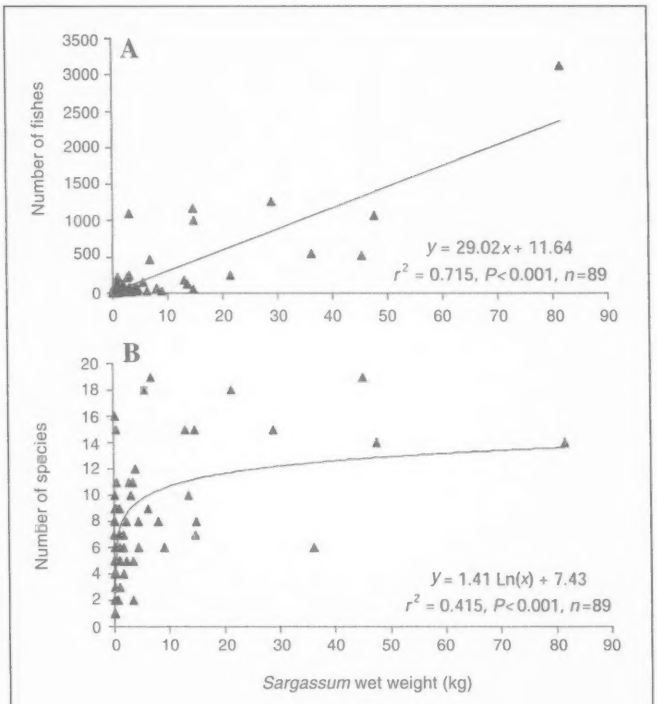

Figure 3

Relationships between number of individuals (**A**) and number of species (**B**) and *Sargassum* wet weight (kg) for all fishes collected with neuston nets in *Sargassum* habitat during summer and fall of 2000–2003 off North Carolina. Note differences in y-axis scale.

Discussion

Pelagic *Sargassum* habitat supports an abundant and diverse assemblage of juvenile fishes, providing structure and protection in relatively barren oceanic surface waters. Juvenile fishes dominate the *Sargassum* community, and the majority of fishes collected in this study from *Sargassum* habitat were comparable in size (<50 mm SL) to those reported from other *Sargassum* studies (Dooley, 1972; Wells and Rooker, 2004). As with seagrass ecosystems

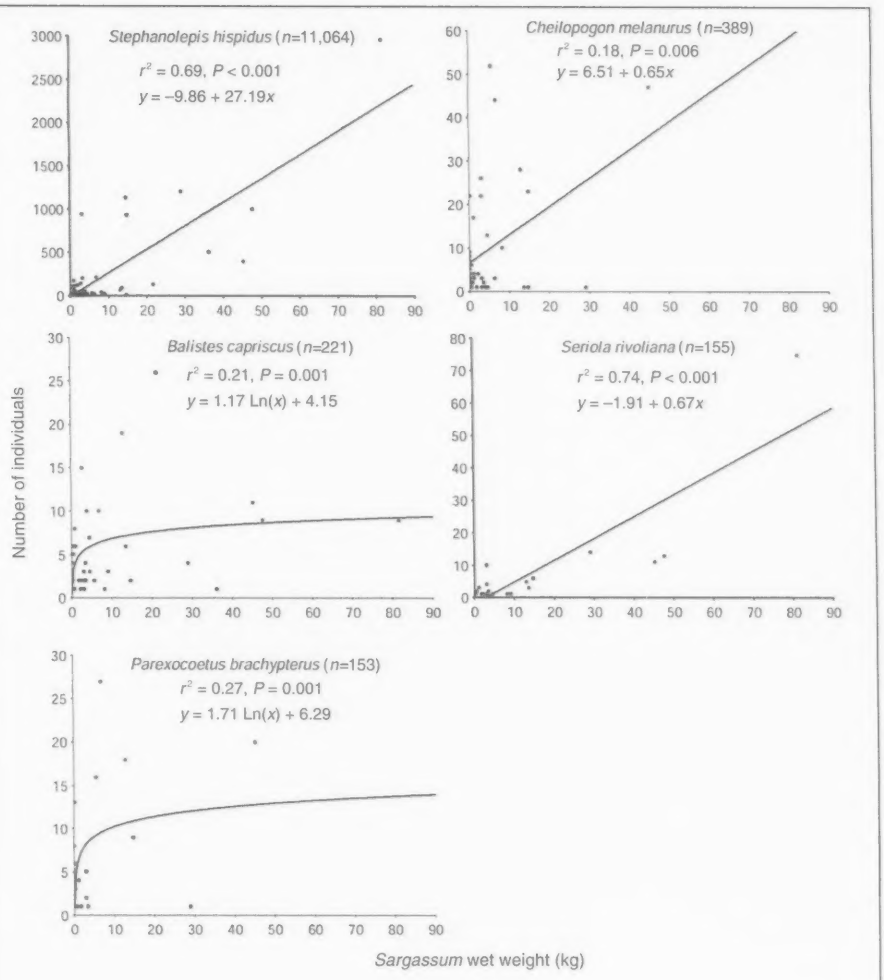
Stephanolepis hispidus (n=11,064)
r² = 0.69, P < 0.001
y = −9.86 + 27.19x
Cheilopogon melanurus (n=389)
r² = 0.18, P = 0.006
y = 6.51 + 0.65x
Balistes capriscus (n=221)
r² = 0.21, P = 0.001
y = 1.17 Ln(x) + 4.15
Seriola rivoliana (n=155)
r² = 0.74, P < 0.001
y = −1.91 + 0.67x
Parexocoetus brachypterus (n=153)
r² = 0.27, P = 0.001
y = 1.71 Ln(x) + 6.29
Number of individuals
Sargassum wet weight (kg)

Figure 4

Relationships between number of individuals and *Sargassum* wet weight (kg) for five abundant fish species collected with neuston nets in *Sargassum* habitat during summer and fall of 2000–2003 off North Carolina. Note differences in *y*-axis scale.

(Stoner, 1983), the strong association of small fishes with *Sargassum* and their behaviors around the algae indicates that this habitat provides shelter from predation. Schooling of *A. monoceros* to mimic floating seaweed (Crawford and Powers, 1953) and the camouflage coloration of juvenile monacanthids, balistids, and other taxa within *Sargassum* fronds help conceal them from predators (Fig. 5, A and D). The increasingly close association of fishes to the floating algae with decreasing fish size further indicates a strong role of the habitat in mitigating predation. Larger fishes, like adult dolphinfishes and jacks, aggregating below the weedlines, appeared to use *Sargassum* primarily during feeding (Dooley, 1972; Moser et al., 1998; this study). *Sargassum* habitat seems to provide an ecological advantage as illustrated by the trend of several species exhibiting larger sizes in *Sargassum* habitat compared to open-water habitat, but it is not clear if this advantage results from better food resources or lack of predation within the algal habitat.

As a result of intensive sampling, the number of fish species known to associate with *Sargassum* habitat in U.S. waters was substantially increased. Eighty fish species were collected in association with *Sargassum* in this study, forty-one percent of which had not been previously reported in association with pelagic *Sargassum*. Mesopelagic fishes spend most of their lives in a habitat lacking structure and have not been reported to seek structured habitats. Thus, the seven species of

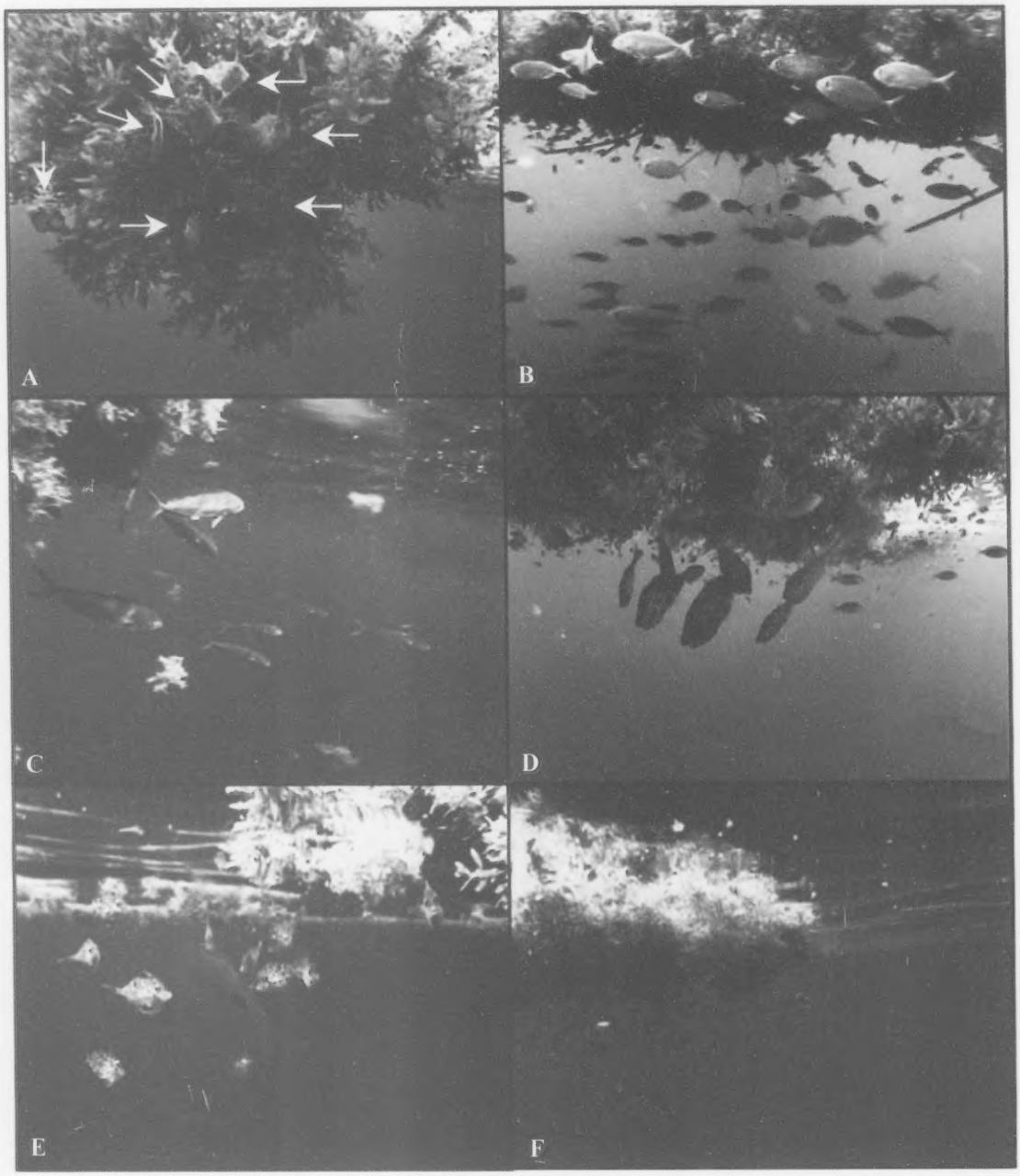

Figure 5

Fishes under a *Sargassum* weedline observed during underwater video recordings, August 1999, off Cape Hatteras, North Carolina. (**A**) Small planehead filefish (*Stephanolepis hispidus*) amongst the *Sargassum*, (**B**) larger, more mobile jacks (carangids) below the *Sargassum*, (**C**) large predators (*Coryphaena hippurus* [dolphinfish]) using *Sargassum* habitat, (**D**) schooling of *Aluterus monoceros* (unicorn filefish) at a 45° angle, (**E**) *Stephanolepis hispidus* pursuing and picking at a lobate ctenophore (*Mnemiopsis leidyi*), and (**F**) edge of a *Sargassum* weedline.

mesopelagic fishes representing three families (Gonostomatidae, Sternoptychidae, Myctophidae) collected with *Sargassum* likely resulted from combinations of their upward diel migrations, upwelling (reported from the Cape Hatteras study area: Lohrenz et al., 2002; Thomas et al., 2002), or convergent currents bringing them into contact with *Sargassum*, rather than the result of attraction to the algae. The associations of many fish species with *Sargassum* appears to be facultative (Dooley, 1972; Wells and Rooker, 2004), and all studies to date have recorded fishes incidentally associated with *Sargassum* that are normally not considered to be structure-associated species. It remains difficult to determine exactly why or how some species use this habitat and the degree to which it influences their life histories.

Despite methodological differences between the studies, patterns of abundance for dominant species collected from *Sargassum* habitat were comparable to those from previous collections off North Carolina, Florida, and in the Gulf of Mexico. *Stephanolepis hispidus* dominated all *Sargassum* collections in all areas, followed closely by *C. crysos* and *B. capriscus* (Dooley, 1972; Wells and Rooker, 2004). *Histrio histrio* was abundant in Gulf of Mexico (Bortone et al., 1977; Wells and Rooker, 2004) and Florida east coast (Dooley, 1972) collections with *Sargassum* but was not abundant in collections off North Carolina (Dooley, 1972; Moser et al., 1998; this study). Because the majority of *H. histrio* are found in the Sargasso Sea and Caribbean Basin (Adams, 1960), their lower abundance off North Carolina may represent a winnowing of the population with northward or westward drift. Dooley (1972) suggested a progressive decrease in fish species richness from Florida to North Carolina and across the Atlantic to the Azores. This decrease in species richness may be an artifact of limited collections off North Carolina and the Azores because our more extensive sampling produced *Sargassum*-related species richness exceeding that reported in other areas. Although based on limited sampling, data indicate that fewer fish species are associated with *Sargassum* habitat in the Sargasso Sea compared with *Sargassum* habitat in the Gulf of Mexico or the Gulf Stream (Fine, 1970; Stoner and Greening, 1984). The great difference between the Sargasso Sea and U.S. continental shelf collections indicates that the majority of the fish fauna recruits to *Sargassum* habitat after the algae are entrained into the Loop Current (Gulf of Mexico) and the Florida and Gulf Stream currents.

The structural complexity of habitats strongly affects fish assemblages. Our open-water samples contained fewer fishes compared with samples containing *Sargassum* habitat. Clearly, fishes that use *Sargassum* habitat also are found in open water without *Sargassum*, but abundance is heavily skewed toward floating structured habitat (Kingsford, 1993). *Stephanopelis hispidus* dominated both habitats but was two orders of magnitude more abundant in *Sargassum* collections. Considering this, and that *S. hispidus* usually occupies structured habitat, it seems likely that the *S. hispidus* collected from open-water habitat may have been displaced by physical disturbance to *Sargassum* mats, or they may have been caught in open water because they had strayed away from the preferred habitat. If so, an even larger difference exists between open-water and *Sargassum* fish communities. The strong fidelity of fishes to floating *Sargassum* habitat is also illustrated by the distinct boundary observed between open-water and *Sargassum* habitats (Fig. 5F). The open water adjacent to the edge of the weedline was unpopulated, compared with the area immediately underneath and within the *Sargassum* where a high density of juvenile fishes was evident. Higher abundances and diversity of fishes in vegetated (versus unvegetated) habitats is a common theme (Weinstein et al., 1977; Orth and Heck, 1980) that results from increased structural complexity (Stoner, 1983). Although fundamental differences exist between *Sargassum* and seagrass ecosystems, fish communities use the two habitats in similar ways. Both habitats are nursery areas for juvenile fishes and support diverse and abundant fish communities. Additionally, the abundance of fishes increases with increasing seagrass density (Orth and Heck, 1980; Thayer and Chester, 1989) and *Sargassum* abundance (Moser et al., 1998; Wells and Rooker, 2004).

Juvenile fishes may seek drifting objects to improve future benthic settlement opportunities (Dempster and Kingsford, 2004), thus facilitating early survival and eventual recruitment to adult populations. Most of the juvenile fishes using *Sargassum* are species that ultimately occupy either inshore benthic reef or complex, structured habitats (demersal) or the open ocean (pelagic). However, the length of time juvenile fishes reside in *Sargassum* and the fates of juvenile fishes after leaving this habitat are unknown. Some fishes remain in the *Sargassum* longer than expected, perhaps because they missed a settlement opportunity. This appears to be the case for some unusually large juveniles (e.g., *Hippocampus* spp., *Mulloidichthys martinicus*, *Kyphosus* spp., *A. saxatilis*, balistids, monacanthids) collected in the present study. Caribbean damselfishes, including *A. saxatilis*, settle between 10 and 12 mm SL (Robertson et al., 1993), yet *A. saxatilis* collected in our study from *Sargassum* habitat were 16–29 mm SL. The dominant *Sargassum*-associated fish, *S. hispidus*, may settle into North Carolina estuarine seagrass beds at 11–40 mm (Adams, 1976; Ross and Epperly, 1985), well below the sizes of some individuals collected offshore in this study. The movement of large quantities of *Sargassum* habitat across the continental shelf as far as the estuaries transports vast numbers of associated juvenile fishes toward other habitats (e.g., seagrass beds, reefs) and probably facilitates recruitment to adult populations.

Young fishes entrained in the Gulf Stream that ultimately have demersal populations, including species using *Sargassum*, have a more uncertain future once they drift north of Cape Hatteras where the Gulf Stream moves offshore (McBride and Able, 1998; Ross et al., 2007). Juveniles of demersal species that do not move from *Sargassum* before reaching the Cape Hatteras area

may 1) exit the Gulf Stream and settle north of North Carolina, 2) continue across the North Atlantic in the Gulf Stream and possibly settle in the eastern Atlantic, 3) complete a circuit of the North Atlantic until they return to the western North Atlantic, or 4) ultimately not contribute to their respective populations (McBride and Able, 1998; Ross et al., 2007). Even though *Sargassum* and associated fishes can be transported into the Middle Atlantic Bight or farther north (Dooley, 1972), the first alternative is unlikely given that most demersal fish species using *Sargassum* are of tropical or warm temperate origins and are not established as adults north of North Carolina (Winge, 1923; McBride and Able, 1998). The second alternative, also suggested by Dooley (1972), seems possible because fifty-three (66%) of the 80 total species collected off North Carolina are established in the eastern Atlantic (Hureau and Monod, 1973a, 1973b), but the link (if any) between these fishes and those in the western Atlantic remains unclear. The third alternative seems least likely because most of the fishes collected in the surface waters do not have larval or juvenile periods long enough to complete a circuit of the Atlantic basin (Ross et al., 2007). It seems likely that many of the fishes remaining in the *Sargassum* north of Cape Hatteras eventually perish. Pelagic species (e.g., carangids, exocoetids, *Coryphaena* spp.) are probably not as restricted and can emigrate from *Sargassum* habitat to open-water habitat over a broader geographic area. Despite the fact that huge numbers of fishes use *Sargassum* habitat in the early life stages, data are lacking regarding its role in transporting juveniles to inshore habitats, in settlement processes, and to what extent *Sargassum*-associated fishes contribute to their respective populations.

Sargassum is an unusual and difficult habitat to sample, and no ideal sampling method has yet been applied. The algae and their medium (water) are in constant motion, and the density and structure of the habitat are constantly changing. One cannot predict exactly when or where *Sargassum* will occur and, unlike static habitats, it cannot be mapped. Thus, it is generally difficult to collect a known number or type of samples from this habitat. Sampling in this study was balanced between day and night, but for the above reasons was not balanced between the two habitats. Sampling the *Sargassum* fauna includes collecting the habitat as well, and the density of the habitat coupled with the three dimensional layering of associated nekton reduce the efficiencies of most sampling gear (especially dip nets). The approach in this study of using a large neuston net to consistently encompass a substantial volume of surface water allowed for large enough samples over a wide range of algal densities.

There is little doubt that *Sargassum* habitat constitutes an important and unique marine ecosystem. It provides a feeding area for many large pelagic fishes, marine mammals, seabirds, and sea turtles. *Sargassum* may enhance early survival of many fishes by protecting them from predation and by concentrating prey, thus providing a unique nursery habitat in an otherwise relatively barren area of the western North Atlantic Ocean. For these reasons, *Sargassum* was designated as essential fish habitat by the South Atlantic Fisheries Management Council. The role of *Sargassum* in transporting juveniles to inshore habitats and subsequent impacts on population recruitment should be investigated.

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Evidence of hook competition in longline surveys

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Abstract—Catch rates from surveys are used as indices of abundance for many fish species. Relative abundance estimates from surveys with longline gear do not usually account for possible effects of gear saturation, which potentially creates competition among fish for baited hooks and misrepresentations of abundance trends. We examined correlations between catch rates of sablefish *(Anoplopoma fimbria)* and giant grenadier (*Albatrossia pectoralis*) and between sablefish and shortraker (*Sebastes borealis*) and rougheye rockfish (*Sebastes aleutianus*) from 25 years of longline surveys in Alaska waters for evidence of competition for hooks. Sablefish catch rates were negatively correlated with giant grenadier catch rates in all management areas in Alaskan waters, and sablefish and rockfish were negatively correlated in five of the six areas, indicating that there is likely competition for hooks during longline surveys. Comparative analyses were done for trawl survey catch rates, and no negative correlations were observed, indicating that the negative correlations on the longline surveys are not due to differing habitat preferences or direct competition. Available adjustments for gear saturation may be biased if the probability of capture does not decrease linearly with baited hooks. A better understanding of each fish species' catch probabilities on longline gear are needed before adjustments for hook competition can be made.

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Catches from longline surveys are used to estimate relative fish abundance for many benthic and pelagic fish species around the world, including: sablefish (*Anoplopoma fimbria*, Hanselman et al., 2006), Pacific halibut (*Hippoglossus stenolepsis*, Clark and Hare, 2006), Greenland halibut (*Reinhardtius hippoglossoides*, Woll et al., 2001; Murua and Cardenas, 2005), sharks (class Elasmobranchia, e.g., Musick et al., 1993; Kohler et al., 1998; Simfendorfer et al., 2002), red snapper (*Lutjanus campechanus*) and yellowedge grouper (*Epinephelus flavolimbatus*) (Cook, 2007), roughhead grenadier (*Macrourus berglax*, Murua and Cardenas, 2005), and many demersal fish species off the Azores Archipelago in the mid-Atlantic (Menezes et al., 2006). In these surveys, it is assumed that catch rate is proportional to abundance, and the probability of catching a fish remains constant even as the number of baited hooks decreases. However, several factors may affect this relationship. Water temperature (Sogard and Olla, 1998a, 1998b; Stoner and Strum, 2004) and feeding history (Løkkeborg et al., 1995; Stoner and Strum, 2004) affect the activity level and feeding behavior of sablefish. The area and strength of the scent plume surrounding bait can also affect the probability of capturing a fish (Løkkeborg et al., 1995). Additionally, in some cases longline gear may become saturated with fish, which potentially creates competition among fish for baited hooks (Murphy, 1960; Rothschild, 1967). Hook competition may reduce the probability of catching a fish as the number of baited hooks decrease, and factors such as temperature and feeding behavior may affect the rate of gear saturation and the intensity of competition for baited hooks.

The National Marine Fisheries Service (NMFS) Alaska Fisheries Science Center (AFSC) conducts annual longline surveys to estimate the relative abundance of major groundfish species in the eastern Bering Sea, Aleutian Islands, and the Gulf of Alaska (Sigler, 2000). The survey is primarily designed to assess sablefish and indices of abundance have been computed since 1979. Recently, catch rates of other groundfish species, such as giant grenadier (*Albatrossia pectoralis*) (hereafter grenadier), and shortraker (*Sebastes borealis*, Clausen, 2006a, 2006b) and rougheye rockfish (*Sebastes aleutianus*, Shotwell et al., 2006), have also been used to assess population levels. Sablefish are the dominant species caught on the longline surveys and most reside in the ~200–1000 m depth range (Sigler, 2000). Sablefish depth range overlaps those of the grenadier (>300 m) and shortraker and rougheye rockfish (~250–500 m), indicating that these species could potentially be competing with sablefish for longline hooks. Also, experimental evidence indicates that sablefish are adept at finding baited hooks on the longline surveys, even when few

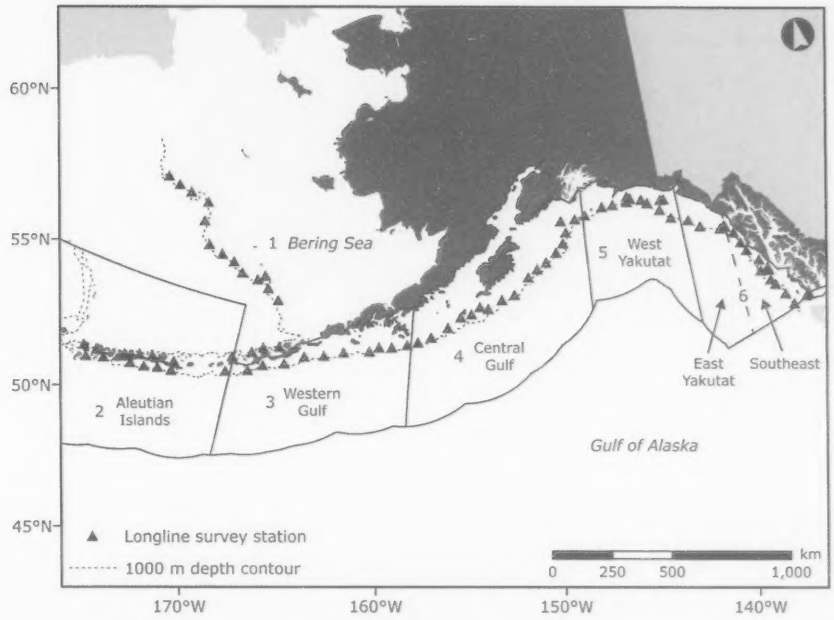

Figure 1

Stations sampled (▲) during the annual National Oceanic and Atmospheric Administration Alaska Fisheries Science Center (AFSC) sablefish (*Anoplopoma fimbria*) longline surveys, 1979–2003 within the six sablefish management areas (1–Bering Sea, 2–Aleutian Islands, 3–Western Gulf of Alaska, 4–Central Gulf of Alaska, 5–West Yakutat, and 6–East Yakutat and Southeast [Alaska]). The AFSC Gulf of Alaska slope trawl survey stations (not shown) are also located along the continental slope above the 1000-m isobath.

remain (Sigler, 2000). Therefore, it is more likely that sablefish are outcompeting grenadier and shortraker and rougheye rockfish for hooks.

Negative correlations between catch rates of two species could be evidence of gear saturation, or could be caused by other factors such as differing habitat preferences or direct competition between species. If the cause of negative correlations is competition for hooks, catch rate trends or depth distribution shifts could be confounded. Additionally, it may not be possible to evaluate the effects of factors such as habitat and other environmental variables until competition effects are taken into account. For this study, we examined the correlations between sablefish and grenadier and between sablefish and shortraker and rougheye rockfish to identify any negative relationships on the longline surveys. We also compared correlations of catch rates from the longline surveys to correlations of catch rates from the AFSC bottom trawl surveys in the Gulf of Alaska to investigate the cause of the relationships on the longline surveys (Britt and Martin, 2001). Because trawl survey catches are not generally susceptible to gear saturation, catches are a reflection of natural fish densities and therefore a good control to compare to longline catch rates (Gunderson, 1993). Although feeding history, water temperature, and other variables could affect the strength of competition between sablefish and grenadier and between sablefish and shortraker and rougheye rockfish, we were unable to control for these variables in this analysis.

Materials and methods

Sampling

AFSC longline surveys The continental slope throughout the eastern Bering Sea, the eastern Aleutian Islands, and the Gulf of Alaska have been systematically sampled each summer since 1979 during the annual AFSC longline surveys. The survey is divided among six sablefish management areas: 1) Bering Sea; 2) Aleutian Islands; 3) Western Gulf of Alaska; 4) Central Gulf of Alaska; 5) West Yakutat; and 6) East Yakutat and Southeast (Alaska). Stations are placed 30–50 km apart and at each station depths from 150 to 1000 m are sampled (Fig. 1). Catches are pooled by management area and an abundance index is computed (Sigler, 2000). The gear on the survey closely resembles gear used by the sablefish fishery in Alaska. The basic unit of gear is a skate; each skate consists of 45 hooks, baited with squid, spaced 2 m apart. At the end of each skate a 3-kg lead ball is

attached to ensure the gear is fished on the bottom. Each station consists of 160 skates set across depth contours from 150 to 1000 m. At each station there are 7200 hooks set over 16 km. The objective of the setting pattern is to evenly distribute sampling effort over the depths that sablefish inhabit. Gear retrieval begins after the gear has soaked for 3 hours, and fish and baited hooks are counted as they are brought aboard. Catch rates are tabulated as the number of fish of each species per skate of gear (no. fish/45 hooks). Bottom depth is recorded every five skates as the gear is hauled. Interpolated depths are assigned to skates that do not have a recorded depth.

Shortraker and rougheye rockfish catches were pooled for this analysis because they are not distinguished from one another during gear retrieval. These species are very similar in appearance and difficult to distinguish, share the same habitat on the upper continental slope, and often are found in fishery hauls (Clausen and Fujioka, 2005). In this article we will refer to both species as rockfish.

AFSC trawl surveys The AFSC groundfish trawl surveys sample the continental shelf and upper continental slope of the Gulf of Alaska at depths to 500 m, and in some years as deep as 1000 m, during the summer (Britt and Martin, 2001; Fig. 1). The surveys follow a stratified random sampling pattern with 49 strata that are categorized by depth, geological area (e.g., gully, slope), and management area (i.e., latitude and longitude). Trawls are hauled at a vessel speed of 3 knots for 15 minutes, or in some years, for 30 minutes, and tows are conducted along a constant bottom depth. An average depth is assigned to each trawl haul. All fish caught on the surveys are enumerated and a catch rate is calculated by dividing the number of fish caught by the area swept by the net (no. fish/km^2).

Analysis

Longline correlations Average catch rates from the 1979–2003 annual longline surveys were calculated by 50-m depth increments to determine the preferred depth range for grenadier and rockfish in each sablefish management area. Analyses were done separately by management area because preferred depth ranges and catch rates have differed in each area for each fish species. The preferred depth ranges of grenadier and rockfish in each area were defined as the range where the average catch rate, in all years, was at least 20% of the highest catch rate. This method resulted in disregarding depths where the catch rate was less that 20% of the average peak catch rate. Our intention was to consider only depths where grenadier or rockfish were prevalent. An average catch rate was then calculated for the preferred depth range for grenadier and rockfish in each management area in each year at each longline survey station. Catch rates were also calculated for sablefish in the grenadier and rockfish preferred depth ranges so that sablefish catch rates could be compared to grenadier and rockfish catch rates. Preferred depth ranges were calculated from longline survey catch rates instead of trawl survey catch rates because there is little fishing effort below 500 m on the trawl surveys. In addition to calculating the average catch rate for the whole preferred depth range, we also calculated the average catch rate by 50-m increments within the preferred depth range in order to make more fine-scale species comparisons.

Before tests for statistical significance, catch rates were transformed by using natural logarithm, square root, or fourth root transformations to help meet assumptions of normality. Pearson's correlation coefficients and tests of significance were calculated. Catch rates calculated as catch per skate did not meet the assumptions of normality due to a prevalence of zeros. Therefore, average catch rates of the preferred depth range by station were used to calculate correlation coefficients.

Longline versus trawl correlations Because longline and trawl data are collected at the same time, areas, depths, and from the same habitats, trawl correlations are a good comparison for longline correlations. In the trawl data, a positive or zero correlation indicates that species do not have differing habitat preferences and that the species do not directly compete with each other. A negative correlation on longline gear and a positive or zero correlation in trawl gear would indicate that the negative correlations in longline gear could be caused by hook competition and not by these other factors.

Trawl catch rates were computed for each haul for each species in each year the trawl surveys occurred (1984, 1987, 1990, 1993, 1996, 1999, 2001, 2003, and 2005). Because depth ranges varied throughout the Gulf of Alaska, the entire depth distribution of grenadier and rockfish throughout the Gulf of Alaska was included in this analysis. The depth distribution was rounded to the nearest 50 m because longline survey catches were already summarized by 50 m increments. For grenadier the range was 300–700 m and for rockfish the depth range was 250–550 m. Catches deeper than 700 m were not included because of the limited trawl survey effort below this depth. Trawl catch rates were also transformed to help meet the assumptions of normality. In one case, namely for the rockfish trawl data, the data could not be transformed to fit a normal distribution, but the transformation was retained because it aided in the visual analysis of the data. In this instance, nonparametric correlation tests, Spearman's ρ and Kendall's τ, were used to calculate correlation coefficients and to test for significance. Longline catch rates were summarized to be comparable with trawl catch rates; average catch rates were computed for the same depth ranges and years at each station.

Results

Longline catch rates by area

Preferred depths of grenadier and rockfish differed by management area (Table 1). Peak catch rates also dif-

Table 1

Preferred depth ranges (m) and average peak number of fish caught per 45 hooks (one skate of gear) for giant grenadier (*Albatrossia pectoralis*) (grenadier) and rockfish (shortraker [*Sebastes borealis*] and rougheye [*Sebastes aleutianus*] rockfish) caught during the National Oceanic and Atmospheric Administration Alaska Fisheries Science Center annual longline surveys in the six management areas, 1979–2003. Preferred depth range is defined as the range in which the catch rate was at least 20% of the average peak catch rate (peak catch).

	Grenadier		Rockfish	
Area	Preferred depth (m)	Peak catch	Preferred depth (m)	Peak catch
Bering Sea	350–1000	5.3	250–550	1.0
Aleutian Islands	400–1000	9.8	250–500	4.1
Western Gulf	400–1000	14.4	250–450	2.8
Central Gulf	350–1000	8.8	300–500	1.8
West Yakutat	350–1000	5.5	250–500	4.2
East Yakutat and Southeast	550–1000	3.5	300–600	4.8

Table 2

Average number of giant grenadier (*Albatrossia pectoralis*) (grenadier) and rockfish (shortraker [*Sebastes borealis*] and rougheye [*Sebastes aleutianus*] rockfish) caught per 45 hooks (one skate of gear) in their preferred depth range in each management area during the National Oceanic and Atmospheric Administration Alaska Fisheries Science Center annual longline surveys, 1979–2003. Preferred depth range is defined as the range in which the catch rate was at least 20% of the average peak catch rate. The average sablefish (*Anoplopoma fimbria*) catches (SB catch) and the average number of baited hooks retrieved in the preferred depth ranges are also shown. Total catch is the sum of the average number of grenadier and sablefish caught per skate or the sum of the average number of sablefish and rockfish caught per skate. Catches and baited hooks are an average from 1995 through 2003 because baited hooks were not counted before 1995.

	Grenadier				Rockfish			
Area	Grenadier catch	SB catch	Baited hooks	Total catch	Rockfish catch	SB catch	Baited hooks	Total catch
Bering Sea	6.6	3.9	14.9	10.5	3.5	8.6	18.9	12.1
Aleutian Islands	15.2	5.0	7.9	20.2	4.3	3.0	9.0	7.3
Western Gulf	18.1	6.4	7.2	24.5	3.0	9.2	4.0	12.2
Central Gulf	9.1	10.5	9.1	19.6	1.4	8.4	10.2	9.8
West Yakutat	3.8	10.6	15.3	14.4	4.3	5.5	19.8	9.8
East Yakutat and Southeast	3.1	11.3	17.9	14.4	3.3	9.5	16.4	12.8

fered substantially by species in each area. For example, in the East Yakutat and Southeast area the preferred rockfish depth range was 300–600 m and the peak catch rate was 4.8 fish/45 hooks, but in the Western Gulf area it was 250–450 m and the peak catch rate was 2.8 fish/45 hooks. The highest grenadier catch rates occurred in the Western Gulf area and were also relatively high in the Central Gulf and the Aleutian Islands areas. The highest rockfish catch rates were in the East Yakutat and Southeast area, West Yakutat, and the Aleutian Islands areas (Table 1).

The number of baited hooks retrieved was similar at grenadier and rockfish preferred depths in each area; however, in the Aleutian Islands, Western Gulf, and Central Gulf areas, the total number of sablefish and rockfish caught at rockfish preferred depths was less than the number of sablefish and grenadier caught at grenadier preferred depths (Table 2). This finding indicates that there were many fish species other than sablefish and rockfish caught at rockfish preferred depths than at grenadier preferred depths.

Longline correlations

In 11 out of 12 cases, there were significant negative correlations between sablefish and grenadier and between sablefish and rockfish catch rates. The correlations between sablefish and grenadier catch rates were negative in all six management areas. Correlations between sablefish and rockfish catch rates were negative in five of the six management areas (Table 3). Sample sizes varied between grenadier and rockfish in some areas because,

Table 3

Correlations between longline catch rates of giant grenadier (*Albatrossia pectoralis*) (grenadier), sablefish (*Anoplopoma fimbria*), and rockfish (shortraker [*Sebastes borealis*] and rougheye rockfish [*Sebastes aleutianus*] combined) caught during the National Oceanic and Atmospheric Administration Alaska Fisheries Science Center annual longline surveys in the six management areas, 1979–2003. The Pearson's correlation coefficient (r) and the P-value associated with the significance of the correlation (P) is shown as well as the sample size (n).

	Grenadier-Sablefish			Rockfish-Sablefish		
Area	r	P	n	r	P	n
Bering Sea	−0.23	0.0006	243	−0.31	<0.0001	243
Aleutian Islands	−0.37	<0.0001	264	0.03	0.6626	264
Western Gulf	−0.38	<0.0001	243	−0.27	<0.0001	243
Central Gulf	−0.36	<0.0001	375	−0.22	<0.0001	368
West Yakutat	−0.38	<0.0001	200	−0.28	<0.0001	200
East Yakutat and Southeast	−0.46	<0.0001	269	−0.43	<0.0001	400

Table 4

Strongest correlations between giant grenadier (*Albatrossia pectoralis*) (grenadier) and sablefish (*Anoplopoma fimbria*) catch rates and between rockfish (shortraker [*Sebastes borealis*] and rougheye [*Sebastes aleutianus*] combined) and sablefish catch rates by 50-m depth increments. Data were collected during the National Oceanic and Atmospheric Administration Alaska Fisheries Science Center annual longline surveys, 1979–2003. The Pearson's correlation coefficient (r), the P-value associated with the significance of the correlation (P), and the sample size (n) are reported. n is often larger for rockfish because in some years the deeper areas, where grenadier reside, were not sampled at each station. Catch rates from the Bering Sea for giant grenadier and sablefish could not be transformed to fit a normal distribution; therefore, no data are presented.

	Grenadier-Sablefish				Rockfish-Sablefish			
Area	Depth (m)	r	P	n	Depth (m)	r	P	n
Bering Sea					350–400	−0.35	<0.0001	199
Aleutian Islands	750–800	−0.44	<0.0001	218	400–450	−0.09	0.1751	218
Western Gulf	450–500	−0.51	<0.0001	239	250–300	−0.31	<0.0001	244
Central Gulf	600–650	−0.51	<0.0001	356	300–350	−0.40	<0.0001	366
West Yakutat	700–750	−0.50	<0.0001	187	350–400	−0.35	<0.0001	199
East Yakutat and Southeast	600–650	−0.44	<0.0001	341	400–450	−0.53	<0.0001	393

at some stations in some years, gear was not set in the preferred depth range for grenadier or rockfish. For example, in the East Yakutat and Southeast area there were 269 station/year combinations for grenadier and 400 for rockfish because gear was not set deep enough for the preferred grenadier depth range at some stations in some years. At certain 50-m depth intervals, within the preferred depth range, correlations were more strongly negative than catch rate correlations for the entire preferred depth range (Table 4).

When sablefish catch rates were high, grenadier and rockfish catch rates were low, and vice versa on the longline surveys. To illustrate an example of these negative correlations, we plotted the transformed catch rates in the East Yakutat and Southeast area (Fig. 2). The 90% density ellipses demonstrated that the trend in the data was negative in both the sablefish and grenadier and sablefish and rockfish scatter plots. Raw, untransformed catch rates also illustrated that the trend between the catch rates was negative. As an example, grenadier and rockfish catch rates were plotted against sablefish catch rates in the East Yakutat and Southeast area (Fig. 3). In both figures, sablefish catch rates were high when grenadier or rockfish were low and vice versa.

When the catch rates of all the stations were averaged within a management area, the negative correlation was evident in the time series. For example, the time series of the average catch rates in the East Yakutat and Southeast area showed that grenadier and rockfish catch rates were higher than average when sablefish catch rates were lower than average and vice versa (Fig. 4). This trend was evident in all areas where there was a negative correlation.

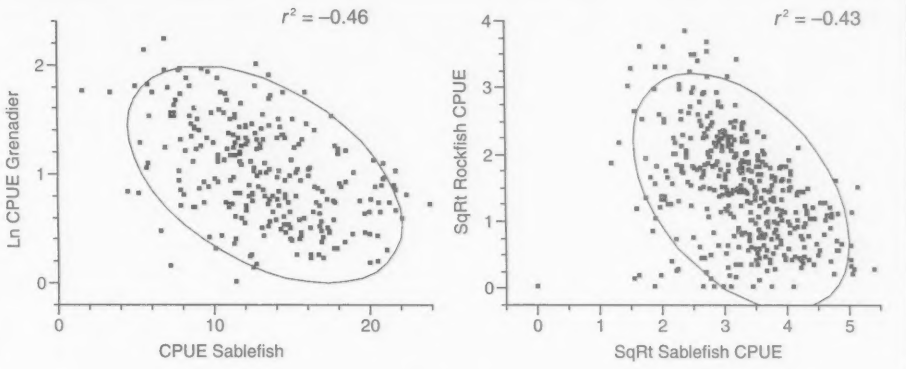

Figure 2

Scatterplots of grenadier (giant grenadier [*Albatrossia pectoralis*]) versus sablefish (*Anoplopoma fimbria*) catch rates and rockfish (shortraker [*Sebastes borealis*] and rougheye [*Sebastes aleutianus*] rockfish) versus sablefish catch rates from the National Oceanic and Atmospheric Administration Alaska Fisheries Science Center annual longline surveys, 1979–2003. Only data from the East Yakutat and Southeast management area are shown as an example. Each data point represents the average number of fish caught per 45 hooks (one skate of gear) at preferred depths at each station per year. Ninety percent density ellipses are included to demonstrate the trend of the relationship. Catch per unit of effort is abbreviated as CPUE. Data transformations are also abbreviated: square root (SqRt) and natural logarithm (Ln).

Table 5

Correlation coefficients (coefficient) between giant grenadier (*Albatrossia pectoralis*) (grenadier) and sablefish (*Anoplopoma fimbria*) catch rates and between rockfish (shortraker [*Sebastes borealis*] and rougheye [*Sebastes aleutianus*] combined) and sablefish catch rates in the Gulf of Alaska. Data were collected during the National Oceanic and Atmospheric Administration Alaska Fisheries Science Center Alaska longline and trawl surveys (1984, 1987, 1990, 1993, 1996, 1999, 2001, 2003, and 2005). The Pearson's correlation coefficient of the relationship between sablefish and either grenadier or rockfish is listed. The coefficients for nonparametric tests (Spearman's ρ and Kendall's τ) are listed for the rockfish and sablefish comparison in the trawl data because these data were not normally distributed. The *P*-value associated with the significance of the correlation tests are shown as well as the sample size (n). The sampling gear is specified as either longline (LL) or trawl (Trawl).

		Grenadier–Sablefish			Rockfish–Sablefish		
Gear	Test	Coefficient	*P*	*n*	Coefficient	*P*	*n*
LL	Pearson's	−0.45	<0.0001	327	−0.24	<0.0001	355
Trawl	Pearson's	0.51	<0.0001	249			
Trawl	Spearman's ρ				−0.08	0.1070	419
Trawl	Kendall's τ				−0.05	0.1102	419

Longline versus trawl correlations

When longline data were summarized in the same way as the trawl data, the correlations between sablefish and grenadier and sablefish and rockfish catch rates were significantly negative (Table 5), just as they were in the analyses that were stratified by sablefish management area. Scatter plots of the joint catch rates of sablefish and grenadier (Fig. 5A) and sablefish and rockfish (Fig. 5B) showed that when catch rates were low for grenadier and rockfish, they were high for sablefish, and vice versa. The 90% density ellipses illustrated the negative trend in the joint catch rates.

Correlation coefficients between sablefish and grenadier catch rates from the Gulf of Alaska trawl surveys were significantly positive and the correlations between sablefish and rockfish catch rates were not significantly different from zero. Because sablefish and rockfish catch rates within the rockfish preferred depth range could not be transformed to fit a normal distribution, nonparametric methods and tests of significances were

used. Both the Spearman's ρ and Kendall's τ had the same result of no significance (Table 5).

Correlations between sablefish and grenadier or rockfish catch rates in trawl gear were either positive or not different from zero. A scatter plot of sablefish and grenadier catch rates showed that there were very few hauls when no sablefish or no grenadier were caught (Fig. 5C). It also demonstrated that when sablefish catch rates were relatively high, grenadier catch rates were high as well. The positive trend in the data is illustrated by the 90% density ellipse. However, the scatter plot of sablefish and rockfish catch rates (Fig. 5D) showed that there were three distinct groups of data: one where there were very few sablefish and many rockfish, one where there were few rockfish and many sablefish, and one where there were high catch rates of both species. The 90% density ellipse showed that the overall trend was neutral. The data for the sablefish and rockfish comparison in trawl gear did not conform to a normal distribution after the data were transformed, although the transformation was retained for ease in viewing the data.

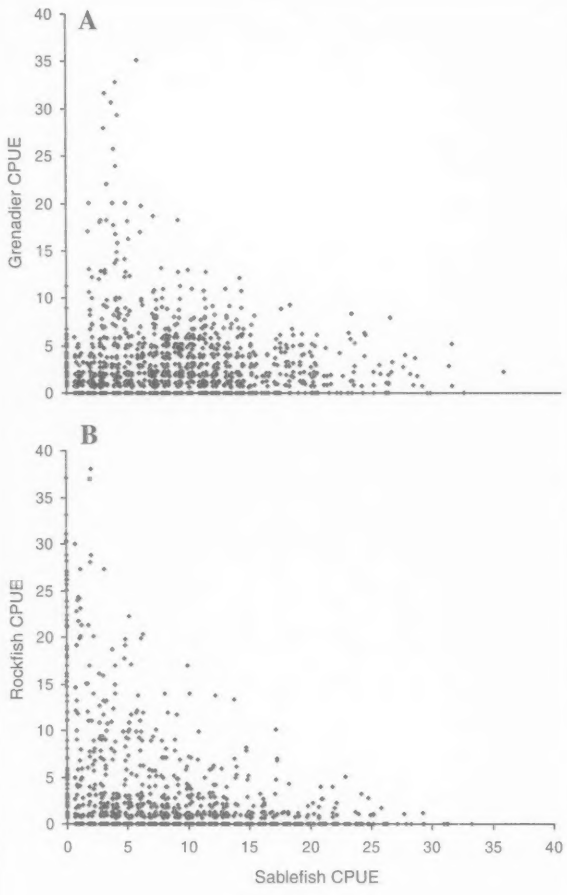

Figure 3

Untransformed catch rates, in number of fish caught per 45 hooks (one skate of gear), for (**A**) grenadier (giant grenadier [*Albatrossia pectoralis*]) versus sablefish (*Anoplopoma fimbria*) and (**B**) rockfish (shortraker [*Sebastes borealis*] and rougheye [*Sebastes aleutianus*] rockfish) versus sablefish caught during the National Oceanic and Atmospheric Administration Alaska Fisheries Science Center annual longline surveys from 1979–2003. Catch per unit of effort is abbreviated as CPUE. Because multiple data points coincide, a random number was added to each point so that they could be spread slightly to illustrate data point frequency. Only catches from the East Yakutat and Southeast management area from 2003 are shown as an example. Only preferred depths were included for grenadier (550–1000 m) and rockfish (300–600 m).

Lengths of sablefish, grenadier, shortraker, and rougheye rockfish were similar in trawl and longline gear. Because large fish were caught by both types of gear, we can assume that both gears are catching adult cohorts of fish. Neither gear appears to be selecting only small juveniles (Fig. 6).

Discussion

Negative correlations on the longline surveys indicated that there was likely competition for hooks between sablefish and grenadier and also possibly between sablefish and rockfish. The catch rate correlations on longline gear between these species were negative in all six of the management areas, with the exception of the correlations for sablefish and rockfish in the Aleutian Islands. The comparison of longline and trawl catch rates in the Gulf of Alaska demonstrated that, in the same areas, depths, and habitats, the relationship between sablefish and grenadier and sablefish and rockfish catch rates on longline gear were negative, but positive for sablefish and grenadier, and neutral for sablefish and rockfish caught in trawl gear. The lack of negative correlations for trawl gear and the presence of negative correlations for longline gear indicated that longline gear was not catching these species in proportion to their abundance. The trawl comparison is important because trawl gear is thought to catch fish in proportion to their abundance, even if the gear may be size selective (Gunderson, 1993). Therefore, hook competition is likely the cause of the negative correlations between sablefish and grenadier catch rates and is also likely one of the factors causing negative correlations between sablefish and rockfish catch rates.

If the cause of the negative correlations on longline gear was due to differing habitat preferences or direct competition, the trawl correlations would also have been negative. In the trawl data, there were very few hauls when zero sablefish or grenadier were caught, demonstrating that sable-

fish and grenadier are found in the same habitats consistently. Additionally, the correlation between catch rates of these species in the trawl gear was positive, also indicating they both use the same habitats. Because catch rates from multiple fixed stations, where the same habitats are sampled each year in several geographic areas for 25 years, consistently show negative correlations, it is unlikely that the negative relationship is due to differing habitat preferences. For example, the time series of catch rates at all stations in the East Yakutat and Southeast area shows that grenadier and rockfish densities are above average when sablefish densities are below average and vice versa (Fig. 4). This result cannot be explained by differing habitat preferences because the same habitats were sampled each year.

Sablefish and rockfish catch rate correlations in the trawl gear showed that there may be three distinct habitat types at rockfish preferred depths, and that negative correlations on the longline surveys may be partially influenced by habitat preferences of sablefish and rockfish at these depths. However, although there appeared to be some different habitat preferences, relatively large numbers of both species were caught in most hauls. Also, nonparametric correlations of trawl catch rates were not significantly different from zero, indicating that any differences in habitat preferences were not great enough to cause negative correlations for the longline gear. Additionally, just as with sablefish and grenadier catch rate trends for longline gear, the rockfish catch rates were above average in years when sablefish were below average, and vice versa, even when the same habitats were sampled each year.

It is also unlikely that sablefish are directly competing with grenadier and rockfish, causing negative correlations. If this were true there would be negative correlations for the trawl gear as well as the longline gear. Also, if sablefish were pushing grenadier or rockfish out of their preferred depth range, grenadier and rockfish catch rates would increase at other depths when sablefish catch rates increased; however, this has not been observed. Or, if their population numbers were actually being depressed because of competition, it would likely take longer time periods for adult populations of long-lived fish species like grenadier and rockfish to rebound.

Although selective differences between longline and trawl gear for species and size have been reported for the Atlantic Ocean (e.g., Hovgard and Riget, 1992; Huse et al., 2000), both gear types caught adult cohorts in these studies. Although gear selectivity of longline and trawl gear in Alaska has not been compared in field studies, all four of the species analyzed in this article were caught in longline and trawl gear in significant numbers. Very few small fish are caught, indicating that both gears catch adult fish (Fig. 6). Moreover, this finding indicates that the same cohorts are being selected by both gear types. Therefore, correlation coefficients between these species in these gear types can be compared fairly.

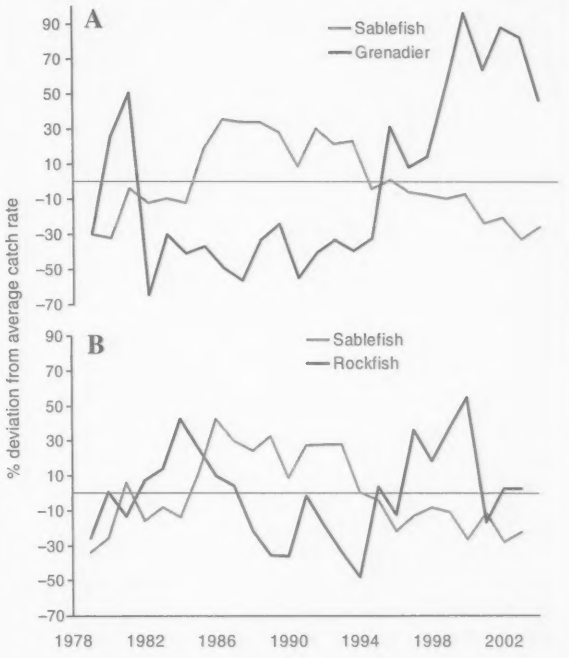

Figure 4

Percent deviation from the average catch rate of the time series for grenadier (giant grenadier [*Albatrossia pectoralis*]), rockfish (shortraker [*Sebastes borealis*] and rougheye [*Sebastes aleutianus*] rockfish), and sablefish (*Anoplopoma fimbria*) caught during the National Oceanic and Atmospheric Administration Alaska Fisheries Science Center annual sablefish longline surveys from 1979 to 2003. (**A**) includes only the grenadier preferred depths and (**B**) includes only the preferred rockfish depths. Data from the East Yakutat and Southeast management area are presented as an example. The lines that intersect the *y*-axis at 0 indicate the average catch rate; anything below this line is lower than average and anything above is higher than average.

Some environmental and fish specific variables, such as feeding history (e.g., Løkkeborg et al., 1995; Stoner and Strum, 2004) may affect the strength of the effect of competition for hooks; however, we could not address these variables. It is possible, however, that at warmer water temperatures fish would have greater metabolic demands and increased hunger, causing more intense competition if prey availability was similar in each scenario (i.e., at colder and warmer temperatures). It is likely that the ability of fish to locate bait and their hunger affects catch rates. To assess the effects of these variables on competition, more laboratory studies of fish behavior would be needed.

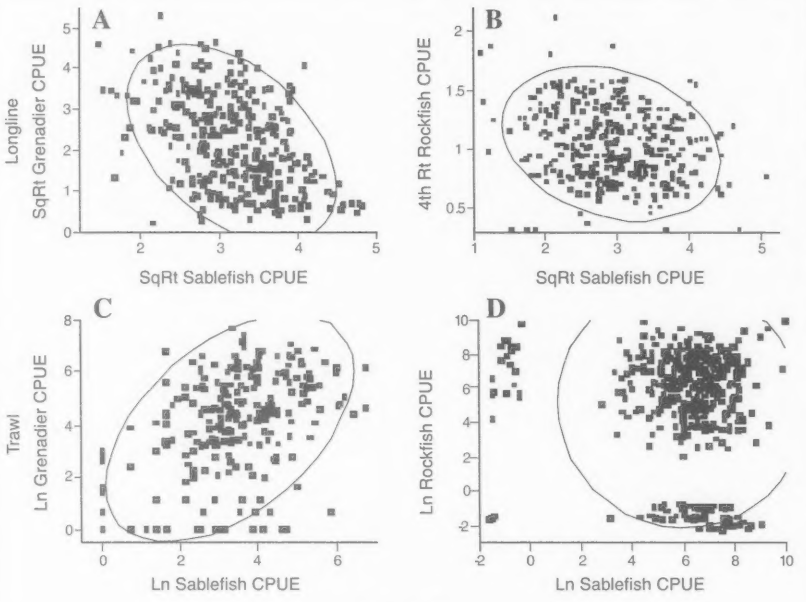

Figure 5

Scatterplots of catch rates from the National Oceanic and Atmospheric Administration Alaska Fisheries Science Center Gulf of Alaska longline and trawl surveys (1984, 1987, 1990, 1993, 1996, 1999, 2001, 2003, and 2005). (**A**) Plot of grenadier (giant grenadier [*Albatrossia pectoralis*]) and sablefish (*Anoplopoma fimbria*) catch rates on longline gear; (**B**) rockfish (shortraker [*Sebastes borealis*] and rougheye [*Sebastes aleutianus*] rockfish) and sablefish catch rates on longline gear; (**C**) catch rates of grenadier and sablefish from the trawl surveys; (**D**) rockfish and sablefish catch rates from the trawl surveys. Ninety percent density ellipses are included to demonstrate the trend. Catch per unit of effort is abbreviated as CPUE. Data transformations are also abbreviated: square root (SQRT), fourth root (4^{th} rt), and natural logarithm (LN).

Effects of competition on grenadier and rockfish catch rates will vary by area because of spatial abundance differences of these species and other groundfish. Negative correlations between sablefish and grenadier catch rates on longline gear varied with the density of sablefish, and the strongest negative relationships were found in the West Yakutat area and East Yakutat and Southeast area where sablefish catch rates and abundances were highest (Hanselman et al., 2006). Negative correlations between sablefish and rockfish catch rates were weaker than the correlations between sablefish and grenadier catch rates. Correlations between sablefish and rockfish catch rates were likely weaker because there were a variety of species caught in relatively large numbers in rockfish preferred depths, such as Pacific halibut (*Hippoglossus stenolepsis*), arrowtooth flounder (*Atheresthes stomias*), and Pacific cod (*Gadus macrocephalus*). In areas where there were many other species caught at preferred rockfish depths (Aleutian Islands, Western Gulf, and Central Gulf areas), the relationship between sablefish and rockfish catch rates was weaker than in other areas. Hence, sablefish and rockfish may compete with multiple species for hooks at rockfish preferred depths and dampen the direct competition with each other. The negative correlations at specific depths where grenadier or rockfish catch rates were highest were even greater than the negative correlations of the entire preferred depth ranges. This finding also shows that hook competition will vary depending on the depth, area, and possibly the abundance of the species of interest as well as other species.

As our data and data from other studies indicate, aggressive predators may out-compete other species for hooks on longline gear (e.g., Skud, 1978; Zenger and Sigler, 1992). For example, the proportion of Pacific halibut caught increased as hook spacing increased because when hooks were widely spaced and less abundant, Pacific halibut out-competed other species for baited hooks (Skud 1978). This experiment also indicates that halibut are caught in proportion to their abundance, while other less aggressive species may not be. Similarly, Zenger and Sigler (1992) and Sigler and Zenger (1994) reported that catches of shortspine thornyhead (*Sebastolobus alascanus*) and grenadier were low in

areas where sablefish catches were high, and vice versa, during the AFSC sablefish longline surveys. They concluded that because sablefish are more mobile and aggressive, they are likely out-competing other species for baited hooks. If competition between less aggressive groundfish and more energetic predators like sablefish is occurring on the AFSC longline surveys, as our data indicate, catch rate trends may not be proportional to actual trends in fish densities.

Directed sablefish experiments have shown that the decrease in probability of catching a sablefish as the number of baited hooks decreases is not linear. However, in existing models where longline catch rates are adjusted for competition, this decrease is assumed to be linear (Murphy, 1960; Rothschild, 1967). In these models, when the number of recovered baited hooks is high, the magnitude of competition is low and model adjustments to catch rates are minimal. Conversely, as fewer baited hooks remain, competition increases, resulting in a greater need for model adjustments. Sigler (2000) documented the time until capture of sablefish on a hook-by-hook basis on longline gear and observed a nonlinear relationship between hooking probability and the number of baited hooks. The probability of hooking a fish remained constant until approximately half of the baited hooks were left; it then decreased steeply toward zero. This indicates that hook competition does not affect sablefish catch rates until fewer than 50% of the baited hooks remain, and that the decrease in hooking probability with a decrease in baited hooks is not linear. In another experiment, Sigler (2000) examined 12- to 42-m hook spacings that represented a condition of only 17% and 5% baited hooks remaining, respectively, and found that sablefish catch per hook decreased only 8%. The hook-spacing experiment showed that there is little decrease in catch probability, indicating that competition affects sablefish catch rates very little and also indicates that the decrease in catch probability does not decrease linearly to zero as the number of baited hooks decreases; it may decrease very slowly until very few baited hooks remain and then drop off quickly. If the effect of baited hooks on catch probability is not linear, as Sigler (2000) showed, results from the Murphy (1960) and Rothschild (1967) competition models used to adjust catch rates would be biased. Assuming a linear relationship, when it is in fact nonlinear, will underestimate relative abundance when fish densities are high, and will overestimate relative abundance when fish densities are low.

Competition for hooks likely occurs during the AFSC longline surveys. Both Skud (1978) and Sigler (2000) found that groundfish catch rates can be affected by hook competition; therefore it is likely that the catch rates of more docile groundfish, such as grenadier and rockfish, would also be affected. Currently gear saturation effects are not taken into account when relative population sizes of groundfish are calculated. Hook timing studies, such as Sigler's (2000), have not been directed at other species caught during the longline surveys. To accurately assess competition effects on grenadier and rockfish, directed experiments on hook spacing or with hook timers would be needed to develop alternate models of the relationship between catch probability and number of baited hooks.

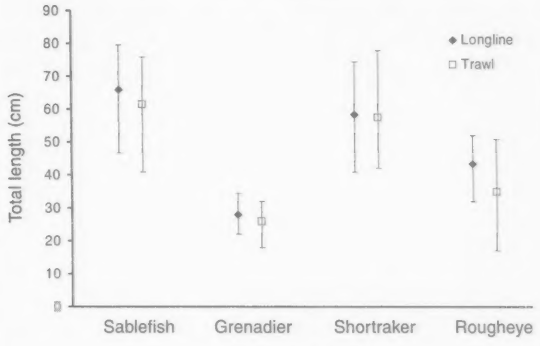

Figure 6

Average total length (cm) and associated 95% confidence intervals for sablefish (*Anoplopoma fimbria*), giant grenadier (*Albatrossia pectoralis*), shortraker rockfish (*Sebastes borealis*), and rougheye rockfish (*Sebastes aleutianus*) caught during the National Oceanic and Atmospheric Administration Alaska Fisheries Science Center longline and trawl surveys in 2003.

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Age validation of Dover sole (*Microstomus pacificus*) by means of bomb radiocarbon

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Abstract—We used bomb radiocarbon (^{14}C) in this age validation study of Dover sole (*Microstomus pacificus*). The otoliths of Dover sole, a commercially important fish in the North Pacific, are difficult to age and ages derived from the current break-and-burn method were not previously validated. The otoliths used in this study were chosen on the basis of estimated birth year and for the ease of interpreting growth zone patterns. Otolith cores, material representing years 0 through 3, were isolated and analyzed for ^{14}C. Additionally, a small number of otoliths with difficult-to-interpret growth patterns were analyzed for ^{14}C to help determine age interpretation. The measured Dover sole ^{14}C values in easier-to-interpret otoliths were compared with a ^{14}C reference chronology for Pacific halibut (*Hippoglossus stenolepis*) in the North Pacific. We used an objective statistical analysis where sums of squared residuals between otolith ^{14}C values of Dover sole and the reference chronology were examined. Our statistical analysis also included a procedure where the Dover sole ^{14}C values were standardized to the reference chronology. These procedures allowed an evaluation of aging error. The ^{14}C results indicated that the Dover sole age estimates from the easier-to-interpret otoliths with the break-and-burn method are accurate. This study validated Dover sole ages from 8 to 47 years.

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The otoliths of Dover sole (*Microstomus pacificus*), a commercially important fish in the North Pacific, are difficult to interpret. Ages derived with the current otolith break-and-burn method have not been previously validated. The age data are important for population modeling and setting the total allowable catch (Stockhausen et al., 2005). The necessity of age validation studies is widely recognized (Beamish and McFarlane, 1983; Campana, 2001) and age validation has become the focus in an expanding number of studies at the Alaska Fisheries Science Center (AFSC) (e.g., Kastelle and Kimura, 2006; Kimura et al., 2006; Hutchinson et al., 2007; Kastelle et al., 2008).

Two methods of Dover sole otolith preparation are used by various agencies. At the AFSC; the Groundfish Program of the Pacific Biological Station, Nanaimo, B.C.; and the Northwest Fisheries Science Center, Dover sole are aged by the break-and-burn method (Chilton and Beamish, 1982) and are estimated to have a maximum age of 54 years. An alternative aging method employed at the Southwest Fisheries Science Center uses transverse thin sections of otoliths (Hunter et al., 1990). The maximum age estimated with thin sections is 58 years (Hunter et al., 1990). The two methods appear to produce similar results but any similarity has not been tested quantitatively, and neither method has been validated.

Workshops on the interlaboratory calibration of methods and on otolith interpretation for determining the age of Dover sole have been held periodically among agencies responsible for the management of this species. However, age validation has not been a focus of these workshops. Because the otoliths of this fish species are small and the species has a relatively long life expectancy, the precision of multiple readings on a sample of otoliths can be poor. The precision measured by percentage coefficient of variation (CV) between two age readers is reported to be 9.64% which is higher than that for most species aged at the AFSC (Kimura and Anderl, 2005). For many North Pacific Pleuronectiformes the CV is under 4% (Kimura and Anderl, 2005). The poor precision (high CV) is an indication of the difficulty in reading otoliths from Dover sole, and indicates that validating the accuracy of the ages is necessary.

The goal of this study was to use bomb-produced radiocarbon (^{14}C) to validate the ages of Gulf of Alaska (GOA) Dover sole determined by the otolith break-and-burn method. Otoliths were selected on the basis of estimated birth year and two descriptive categories: 1) otoliths with uniform growth zones that were easy to enumerate, and 2) otoliths with growth zones that were difficult to enumerate. The first category was used to validate general aging criteria and the second was used to help

determine otolith interpretation. Otolith material corresponding to a time near birth was isolated by extracting the core up to the first 3 years and measured for its ^{14}C content. We analyzed the $\Delta^{14}C$ from the first category of otolith cores using statistical methods first reported by Kastelle et al. (2008).

Bomb radiocarbon age validation has been used on an increasing number of species and is considered one of the best methods to confirm the accuracy of fish ages (Campana, 2001). Recent uses in the North Pacific include that on the white shark (*Carcharodon carcharias*) (Kerr et al., 2006), quillback rockfish (*Sebastes malinger*) (Kerr et al., 2005), canary rockfish (*S. pinniger*) (Piner et al., 2005; Andrews et al., 2007), bocaccio rockfish (*S. paucispinis*) (Andrews et al., 2005; Piner et al., 2006), Pacific halibut (*Hippoglossus stenolepis*) (Piner and Wischniowski, 2004), and Pacific ocean perch (*S. alutus*) (Kastelle et al., 2008).

Radiocarbon fish age validation relies on a time reference provided by production of ^{14}C from atomic bomb testing. The above-ground testing of atomic bombs that introduced ^{14}C into the atmosphere and marine environment began in the 1950s and continued into the 1960s (Kalish, 1993; Nydal, 1993). This caused a rapid increase in marine ^{14}C lasting through about 1970—an increase that is recorded in calcified marine organisms and otoliths and provides a necessary time reference. To validate ages from a "validation species" (in this case Dover sole), a ^{14}C "reference chronology" is used, where the exact time frame of the ^{14}C increase is considered known. Two reference chronologies have been developed for the North Pacific Ocean: one from Pacific halibut (Piner and Wischniowski, 2004) and one from yelloweye rockfish (*S. ruberrimus*) (Kerr et al., 2004). The posited birth years for the validation species are calculated from ages estimated by otolith growth zone counts and date of collection. Specimens representing the validation species are chosen such that the range of posited birth years spans the period of rapid marine ^{14}C increase. Otolith core material deposited in the first one or two years of life from the validation species is analyzed and each core provides one ^{14}C data point. To evaluate the ages, the ^{14}C from the cores of the validation species is plotted with respect to the posited birth years and compared to the known ^{14}C values in the reference chronology. If there is a timing difference between the ^{14}C increase in the validation species and the reference chronology, then the estimated ages of the validation species are often assumed to be in error. Alternatively, if a timing difference is not present, the ages from the validation species are considered accurate. In a recent bomb radiocarbon age validation study of Pacific ocean perch, a series of new procedures was used to compare the ^{14}C measurements in the validation samples to the reference chronology (Kastelle et al., 2008). We used the same methods here—purposely biasing the ages to be validated by ±0, 1, 2, and 4 years; standardizing the validation sample ^{14}C values to the reference chronology; and evaluating the residuals between the validation samples and the reference chronology to see if inaccuracies in the age estimates were present.

There are two important assumptions when validating fish ages with the bomb radiocarbon method (Campana and Jones, 1998; Piner and Wischniowski, 2004; Piner et al., 2005; Kastelle et al., 2008). Assumption 1 is that the validation species must be biologically and environmentally similar to the species in the reference chronology during the first years of life. If both species are receiving their ^{14}C from the same sources, then the magnitude and timing of the ^{14}C increase should be similar (Andrews et al., 2007). A reference chronology based on the same species as that being investigated is best, and occasionally available (Campana, 1997; Campana et al., 2002; Piner and Wischniowski, 2004). Assumption 2 requires that the otolith core used for each ^{14}C analysis be uncontaminated and that it constitute a closed system. Therefore, an accurate extraction of the core without contamination from other carbon sources or different years is necessary. Dover sole otoliths presented a unique challenge in this regard because of their small size. For further information regarding radiocarbon age validation studies, one can consult the earlier mentioned studies from the North Pacific along with Kalish (1993, 1995) and Campana (1997).

Materials and methods

Otolith selection and coring procedures

The Gulf of Alaska (GOA) Dover sole otoliths used in this study were collected either during AFSC survey cruises or by AFSC fishery observers aboard commercial vessels. The survey cruises took place in 1984 and 2005; the otoliths were removed from the fish at sea, stored in a glycerin and thymol mixture, and archived for future age determination. The specimens collected from commercial harvests were caught in 1998 and treated similarly, except they were first stored dry for up to 3 months before storage in a glycerin and thymol mixture. The glycerin and thymol mixture is not expected to be a contaminant in otolith ^{14}C measurements (Campana et al., 2003).

After the archival period, the otoliths were aged at the AFSC for stock assessments. The initial ages were determined by the break-and-burn method (Chilton and Beamish, 1982) with the blind-side otolith. Assumed annual growth zones were counted by enumerating the translucent zones. The otolith growth over the course of one year is assumed to consist of an opaque zone and a translucent zone. After growth zones were read, the otoliths were archived again for varying durations up to 14 years. Samples where the initial age estimate placed the birth year near the era of marine ^{14}C increase were re-examined by age readers experienced in the interpretation Dover sole otolith growth zones, and considered for possible ^{14}C measurement. In the re-examination process otoliths were re-aged to assign a "final age" and were placed into two subjective categories based on the ease of interpretation of the growth zones:

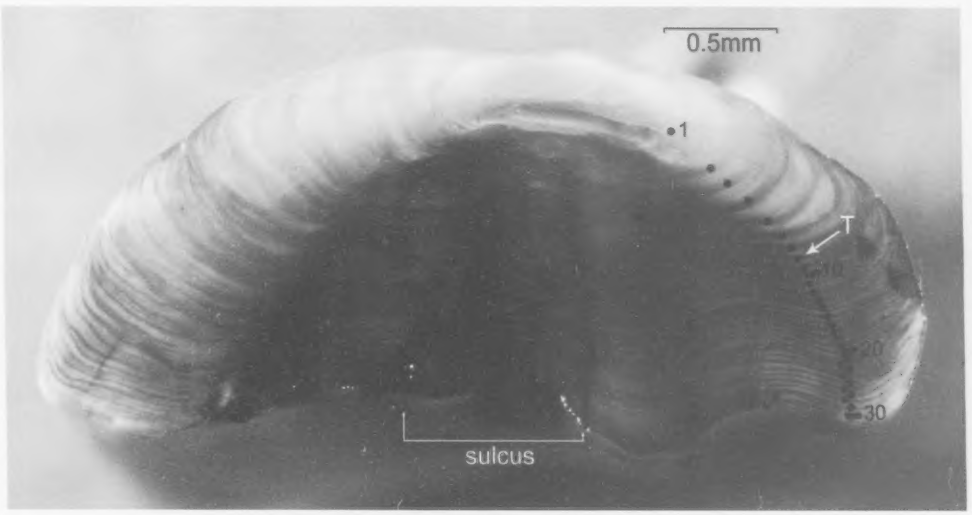

Figure 1

Category-1 otolith (specimen number 82) from Dover sole (*Microstomus pacificus*) treated by the break-and-burn method. The series of dots indicate the translucent zones that were counted to estimate a final age of 32 years. The "T" arrow points to the transition region from fast early growth to slower later growth. The sulcus region of the otolith is indicated as a reference point.

1 Category-1 otoliths had clear growth zones that were easy to interpret, in that most of the translucent zones appeared with minimal or no splitting in the proximal growth axes in at least one region (dorsal or ventral) on the break-and-burn cross section (Fig. 1). Splitting is defined as the branching of a single translucent zone into two or more translucent zones. Typically the translucent zones were spaced evenly, but with decreasing intervals, as the fish became older. Some samples in this category may have presented interpretative options, and different reading axes in the cross section could be chosen, but typically only small differences in age (of 1 or 2 years) would result.

2 Category-2 otoliths had growth zones that were difficult to interpret and that made these fish difficult to age. Many translucent zones had obvious splits and uneven spacing (Fig. 2). Widely different ages could be generated depending upon the interpretation chosen and which reading axis was used.

A sample selection process for ^{14}C measurement occurred after re-examination of the ages, and this selection process relied on two factors. First, in category 1 our intention was that the initial age and final age should agree within 3 years. For otoliths in category 2, which are more common for Dover sole than are category-1 otoliths, we did not use age agreement as a selection factor. Second, the estimated birth years, which were based on the final ages, had to be evenly distributed from about 1951 to 1977, to bracket the era of marine ^{14}C increase. For several specimens in category 1, the first factor was relaxed to evenly populate the years of marine ^{14}C increase. This process provided 43 specimens for $\Delta^{14}C$ analysis: 38 otoliths in category 1 and 5 in category 2 (Table 1). The range in catch years from 1984 to 2005 generated a large range in Dover sole ages for potential validation. In the remainder of this article, unless specified differently, the ages referred to are these final ages.

For the selected Dover sole specimens, the otolith core was extracted from the remaining whole eyed-side otolith for ^{14}C analysis. The core represented material deposited only in the first three years of life. Previous studies have often used a 1-year core (e.g., Campana, 1997), but that was not possible with Dover sole because their otoliths are very small, therefore, the minimum mass required for ^{14}C analysis mandated a 3-year core. For the otolith coring procedure we used a Buehler® EcoMet® (Buehler Ltd., Lake Bluff, IL) grinder with 320 grit wet or dry sandpaper to first remove otolith material on the proximal surface (the main growth axis in older otoliths). On some otoliths a small amount of material was also removed on the distal surface. Next, the grinder was used to remove material on the perimeter, in the anterior-posterior and dorsal-ventral axes, beyond the third year. After the exterior layers were removed with this procedure, the location of the third year's growth zone in each otolith became easier to see, and its location served as a primary guide in the coring

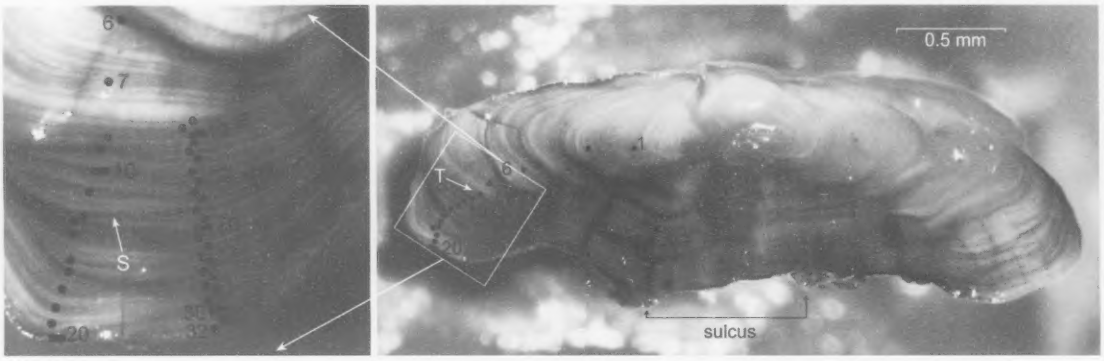

Figure 2

Category-2 otolith (specimen 225) from Dover sole (*Microstomus pacificus*) treated by the break-and-burn method. The "T" arrow points to the transition region from fast early growth to slower later growth. In the enlarged section, the two series of dots represent different options of interpreting the translucent zones; the final age of 20 years and the maximum age of 32 years are shown. The "S" points to a translucent zone that splits to form two translucent zones. The sulcus region of the otolith is indicated as a reference point.

process. Each core was tailored to the size and shape of the apparent third year. A secondary guide for the coring process was the weight and size of otoliths from 3-year-old fish in the 2005 survey collection, and an additional survey collection (not used for ^{14}C measurements) from 2003. These otoliths from 3-year-old fish had an average weight of 9.5 mg (±0.4 mg standard error) and an average size of 2.41×3.84×0.68 mm (n=21). The core would occasionally break into several pieces as material was removed, but all salvageable pieces were retrieved and used. The core size was recorded for all intact cores and compared to otoliths from young fish. Finally, the cores were cleaned in an ultrasonic cleaner, dried, weighed, and then stored in acid-washed vials before ^{14}C analysis.

^{14}C analysis

The ^{14}C and ^{13}C of Dover sole otolith cores were measured at the National Ocean Sciences Accelerator Mass Spectrometry Facility at the Woods Hole Oceanographic Institution, Woods Hole, MA. Samples were treated at the Woods Hole Oceanographic Institution with a routine acid hydrolysis procedure to produce a graphite target, and analyzed with accelerator mass spectrometry. We report results as $\Delta^{14}C$, which is defined in Stuiver and Polach (1977) as the relative difference between an international standard (base year 1950) and sample activity. The $\Delta^{14}C$ is normalized to 1950 and corrected for isotopic fractionation with the $\delta^{13}C$ measurement and normalized to a $\delta^{13}C_{VPDB}$ value of –25 ‰.

To evaluate the Dover sole ages, we compared the otolith $\Delta^{14}C$ results from the otolith cores with the Pacific halibut reference chronology using several procedures. Initially, the $\Delta^{14}C$ from the Dover sole otolith cores in both categories was plotted along with a loess (locally weighted least squares) smoothed curve of the $\Delta^{14}C$ in Pacific halibut. All loess-smoothed curves in this study were fitted by using Splus (Insightful Corp., Seattle, WA) (Chambers and Hastie, 1992) with a span of 2/3 and a degree of 2. To further analyze the $\Delta^{14}C$ results in comparison with the reference chronology, we used three procedures first introduced by Kastelle et al. (2008). The first procedure was to purposely bias the category-1 ages by 0, ±1, ±2, and ±4 years, generating seven sets of ages. For each of these seven sets of biased ages, posited birth years were calculated and a sum of squared residuals (*SSR*) between the $\Delta^{14}C$ in Dover sole otoliths and the loess smoothed data in the Pacific halibut reference $\Delta^{14}C$ chronology was calculated. The smallest of the seven *SSR*s indicated which purposely biased set of ages represented the best fit, and thereby indicated if an overall aging error in the Dover sole ages existed. We call this procedure the "unstandardized" analysis.

In the second procedure used to investigate the accuracy of Dover sole ages in category 1 we performed a "standardization" of the measured $\Delta^{14}C$ values (Kastelle et al., 2008). This is a linear transformation of the $\Delta^{14}C$ values in the Dover sole otolith cores which removes any difference in scale between the validation sample $\Delta^{14}C$ measurements and the reference chronology. It does not change the timing of the validation sample $\Delta^{14}C$ values or their relative magnitude. For this standardization, let $\{v_{y[j]}\}$ be the series of j=1, ..., n validation observations of $\Delta^{14}C$, where $y[j]$ refers to the year core j was formed. We defined the standardized series for $\{v_{y[j]}\}$ as $\{\hat{v}_{y[j]}=(v_{y[j]}+\mu)/\sigma\}$, where μ and σ can be estimated by a least squares fit (i.e., by minimizing the *SSR*) to the loess-smoothed curve of the reference chronology data set $\{l_{y[j]}\}$:

Table 1

Age estimates (in years) and radiocarbon measurements from Dover sole (*Microstomus pacificus*) otoliths collected from the Gulf of Alaska. Category 1 represents easy-to-age specimens and category 2 represents difficult-to-age specimens. Estimated birth year was calculated from final age and known capture date. The final ages are re-evaluated ages undertaken in the current study after initial aging for stock assessments. Postmeasurement min.–max. ages were generated after the radiocarbon values were known. Results are reported as $\Delta^{14}C$ (which is defined in Stuiver and Polach [1977] as the relative difference between an international standard [base year 1950] and sample activity), $\Delta^{14}C$ 95% confidence intervals (CI) were derived from accelerator mass spectrometry error, and the $\delta^{13}C$ measurements were used to correct for natural effects of isotopic fractionation.

			Age estimates (years)				
Specimen no.	Category	Estimated birth year	Final	Postmeasurement min.–max.	$\Delta^{14}C$ (‰)	$\Delta^{14}C$ 95% CI	$\delta^{13}C$ (‰)
3	1	1972	12		80.7	7.2	–1.34
11	1	1965	33		43.8	6.8	–1.86
17	1	1968	30		80.9	8.4	–1.99
18	1	1963	35		27.2	7.8	–1.6
20	1	1974	24		57.6	7.0	–1.5
21	1	1957	41		–86.3	6.2	–0.97
24	1	1958	47		–68.0	6.0	–1.65
34[1]	1	1953	45		–108.1	5.6	–0.97
36	1	1974	10		80.3	7.2	–1.77
48	1	1959	39		–64.9	6.6	–1.12
49	1	1969	29		81.5	7.4	–1.06
54	1	1957	27		–76.2	6.6	–0.98
66	1	1960	24		–65.2	8.0	–1.11
73	1	1964	20		49.2	10.2	–2.26
82	1	1966	32		68.0	7.2	–2.03
84	1	1977	21		36.2	8.0	–1.88
85	1	1966	32		86.4	8.2	–1.26
89	1	1959	39		–84.7	7.0	–1.14
102	1	1967	31		73.7	8.2	–1.12
130	1	1964	34		38.3	7.8	–0.76
138	1	1969	15		72.7	6.6	–1.4
143	1	1953	31		–108.2	5.6	–0.7
157[1]	1	1970	28		57.5	6.6	–0.95
159	1	1976	8		72.6	10.0	–1.88
160	1	1962	36		21.4	7.6	–2.07
210	1	1951	33		–109.5	5.4	–0.57
273	1	1963	21		9.5	8.6	–2.06
302	1	1975	9		64.9	9.8	–1.53
33	1	1959	39		–52.7	6.2	–1.92
34[1]	1	1963	35		41.5	9.0	–1.6
35	1	1970	28		54.3	6.4	–1.17
52	1	1969	29		83.6	8.4	–1.66
71	1	1955	43		–86.7	5.6	–0.71
157[1]	1	1974	10		62.2	6.8	–1.06
201	1	1955	29		–104.3	6.4	–1.32
257	1	1966	18		38.0	6.8	–1.34
271	1	1972	12		67.3	8.2	–1.41
310	1	1967	17		52.3	7.2	–2.78
95	2	1957	48	37–50	–120.1	5.8	–1.59
146	2	1958	26	16–26	75.7	10.2	–0.84
188	2	1953	52	37–59	–105.1	6.8	–0.92
225	2	1964	20	17–32	56.9	7.2	–1.33
317	2	1961	23	19–23	54.7	10.2	–1.41

[1] Specimens with repeated identification numbers do not represent repeat measurements but are different specimens with the same identification numbers from different collection years.

$$SSR = \sum_j (\hat{v}_{y[j]} - l_{y[j]})^2$$

$$= \sum_j [((v_{y[j]} + \mu)/\sigma) - l_{y[j]}]^2.$$

An iterative process to estimate μ and σ can be found in Kastelle et al. (2008). Again, to evaluate for aging error, we purposely biased the ages by 0, ±1, ±2, and ±4. For each of these seven sets of ages, we estimated μ and σ, standardized the validation sample $\Delta^{14}C$ observations with $\{\hat{v}_{y[j]}\}$, and calculated the *SSR*. As before, the smallest *SSR* indicated the best fit to the reference chronology and indicated if any overall aging bias (error) existed. It should be noted that if μ and σ are not estimated, but instead are set to $\mu=0$ and $\sigma=1$, this process becomes the unstandardized procedure described earlier.

The third and final method we employed was estimation of confidence intervals around the loess-smoothed reference chronology (Kastelle et al., 2008). For the estimated confidence intervals, simultaneous Bonferonni statistical inference was used (Miller, 1966) that calculates simultaneous (α=0.01) confidence intervals whose width is dependent on the number of distinct years at which comparisons are made between the reference chronology and validation samples, and on the variability in the reference chronology. As an aid in our comparison between the standardized Dover sole otolith $\Delta^{14}C$ values and the Pacific halibut reference chronology, not only were the *SSR*s evaluated, the standardized $\Delta^{14}C$ values were also viewed graphically when plotted against the posited birth years. This comparison generated seven plots, one for each purposeful bias, and included the loess-smoothed Pacific halibut reference chronology with confidence intervals.

The category-2 otoliths were re-examined again after the $\Delta^{14}C$ results were known. The goal of this re-aging process was to use the $\Delta^{14}C$ results as a guide for refining current otolith aging criteria. To facilitate this process, the $\Delta^{14}C$ in the category-2 otoliths was plotted with a loess-smoothed curve of category-1 results. This process allowed the age reader to learn how difficult-to-interpret otoliths are best aged. In this last re-aging process a minimum and maximum age were estimated for the category-2 otoliths.

The 3-year core in Dover sole otoliths needed to be taken into consideration when analyzing the results. We assumed a mid-point of approximately 1.5 years for core deposition because the core represents material from the first 3 years of life. This means that we assumed linear otolith growth during the first three years of life. Linear otolith growth may not be accurate, but was assumed for simplicity and because any error from this assumption is trivial. The Pacific halibut reference chronology is based on material from only the first year of life; therefore, we assumed a mid-point of 0.5 years for core deposition. These assumptions mandated an approximate allowance of a (1.5–0.5 =) 1.0-year shift in the comparison of the Dover sole results with the Pacific halibut reference chronology.

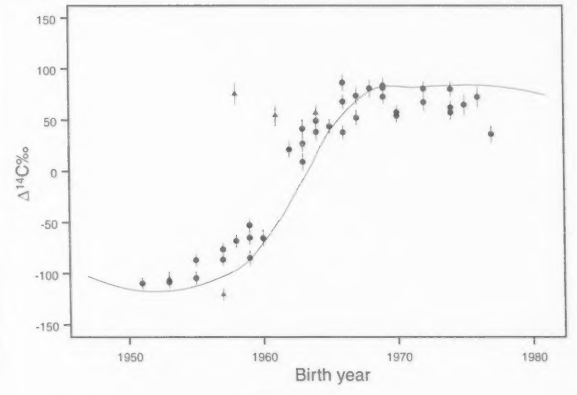

Figure 3

$\Delta^{14}C$ ‰ in otolith cores plotted against birth year of Dover sole (*Microstomus pacificus*), by category 1 (●) and category 2 (▲), with a 95% confidence interval shown for each point. Two category-1 specimens are overlapping at –108 $\Delta^{14}C$ and birth year 1953. The line (——) is a loess-smoothed curve of the Pacific halibut (*Hippoglossus stenolepis*) reference chronology (Piner and Wischniowski, 2004).

Results

Otolith selection and coring procedures

The selection process generated specimens for which final ages agreed with initial ages and was followed by successful coring. In all but three of the specimens in category 1, the agreement between the initial age and final age was within 3 years. In this category, the maximum final age was 47 years, and the minimum age was 8 years (Table 1). In category-2 specimens, four out of five had discrepancies of over 3 years between the initial age and final age. The percentage CV (Kimura and Anderl, 2005) between initial ages and final ages in both categories was 4.21%. The average core weight across all categories was 5.4 mg (±0.2 mg standard error) and the average size was 1.93×2.97×0.49 mm, which was smaller than the guide provided by the 3-year-olds described earlier.

^{14}C analysis

The $\Delta^{14}C$ in Dover sole otolith cores from category 1 followed the expected general pattern of initial low ^{14}C before atmospheric testing followed by an increase synchronous with testing. It displayed a rise in about 1955 (from below –100‰) and peaked in 1966 at over 85‰ (Table 1, Fig. 3). This trend in Dover sole radiocarbon

Table 2

Sum of squared residuals (*SSR*) between $\Delta^{14}C$ in category-1 Gulf of Alaska Dover sole (*Microstomus pacificus*) otoliths and loess-smoothed curve of the Pacific halibut (*Hippoglossus stenolepis*) reference chronology (Piner and Wischniowski, 2004). Results are tabled by unstandardized (No) or standardized (Yes) $\Delta^{14}C$ values. The μ and σ are coefficients in the linear standardization defined for the series $\{v_{y[j]}\}$ as $\{\hat{v}_{y[j]}=(v_{y[j]}+\mu)/\sigma\}$, where μ and σ were estimated by a least squares fit to the loess-smoothed curve of the reference chronology data set, or set to 0 and 1 respectively when unstandardized. When standardized, *SSR* was minimized with respect to μ and σ. Age bias was applied to each final age estimate such that –4 represents younger ages and +4 represents older ages.

$\Delta^{14}C$ Standardized to reference	Parameter	Age bias (years) –4	–2	–1	0	+1	+2	+4
No	μ	0	0	0	0	0	0	0
	σ	1	1	1	1	1	1	1
	SSR	40,429	13,179	11,651	20,140	38,281	66,696	153,202
Yes	μ	28.47	9.59	1.63	–6.17	–13.97	–22.80	–45.09
	σ	1.10	0.94	0.91	0.90	0.92	0.95	1.11
	SSR	15,544	7969	9533	16,834	29,191	45,706	84,612

values parallels those seen in the Pacific halibut reference chronology but the values appear to be shifted earlier in time by 1 or 2 years. Also, from 1969 on, the Dover sole values are mostly lower than those in the reference chronology (Fig. 3).

In the analysis of the category-1 ages, a purposeful bias of –1 or –2 years provided the lowest *SSR*s. In the unstandardized procedure, where the ages were purposely biased –1 years, the *SSR* was smallest at 11,651 (Table 2). In the standardized procedure when the ages were purposely biased –2 years, the *SSR* was smallest at 7969, where $\mu=9.59$ and $\sigma=0.94$ (Table 2). For the standardized analysis, a series of plots is presented, one plot for each of the seven sets of purposely biased ages (Fig. 4). Both the overall time shift and the low bias after 1969 were removed by the standardization procedure when the purposeful age bias was –2 years (Fig. 4B). Also, at this purposeful age bias and standardization, the Dover sole validation specimens were all within the 99% confidence intervals around the loess-smoothed reference chronology.

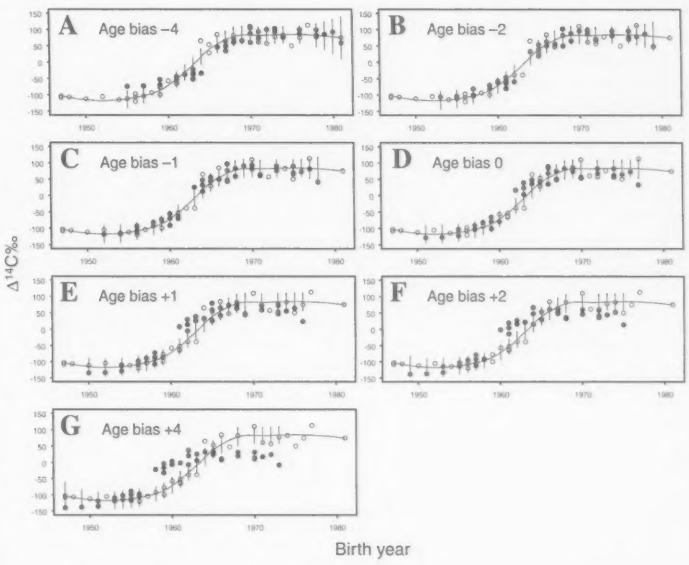

Figure 4

Series of seven plots for $\Delta^{14}C$ ‰ in otolith cores of Dover sole (*Microstomus pacificus*) category-1 (●) where the $\Delta^{14}C$ is standardized and each plot, (**A**) through (**G**), corresponds to a purposeful age bias of –4 to +4 years, respectively, with a loess-smoothed curve (——) of the Pacific halibut (*Hippoglossus stenolepis*) reference chronology (○) (Piner and Wischniowski, 2004) and 99% simultaneous confidence intervals around the mean smooth.

The general difficulty in aging category-2 specimens is evident in the $\Delta^{14}C$ results that are not synchronous with those from the category-1 specimens. The range of possible ages for category-2 specimens (Table 1) is shown by horizontal bars in Figure 5. Two of the three younger category-2 specimens were likely over-aged because only the right end of the horizontal bar is close to the values for the other specimens or the values of the loess-smoothed curve of category-1 specimens. Therefore, for these three specimens the choice of a younger age is more accurate. In

the two older category-2 specimens, the results are less definitive because the $\Delta^{14}C$ values fall in the stable pre-bomb era. Figure 2 is an example of how a category-2 otolith may be aged given the different possible interpretive options.

Discussion

Age validation

This study is the first published age validation for GOA Dover sole. Ages in the range of 8 to 47 years were validated. Accurate ages were indicated by both the unstandardized and standardized $\Delta^{14}C$ results for category 1 when a purposeful age bias of -1 or -2 years produced the lowest *SSR*s. The shift of approximately 1 year (due to core size, as explained earlier) was expected, and when considered with the purposeful age bias and lowest *SSR*s, indicated that ages estimated by the break-and-burn method were accurate. All category-1 specimens were within the 99% simultaneous confidence intervals on the reference chronology (Fig. 4B) and this result also provides strong evidence for accurate ages.

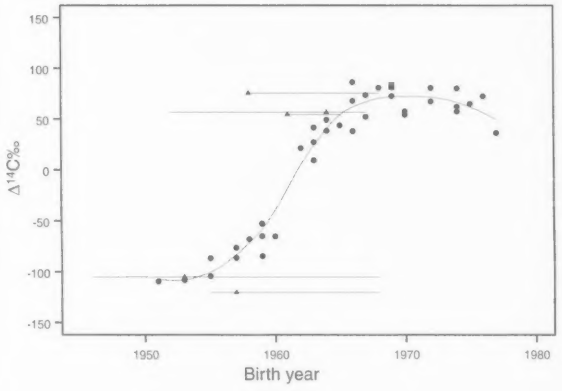

Figure 5

$\Delta^{14}C$ ‰ in otolith cores of Dover sole (*Microstomus pacificus*) plotted against birth year, by category 1 (●) and category 2 (▲). Horizontal lines on category-2 specimens representing the minimum and maximum postmeasurement age, and a loess smoothed curve (——) has been fitted to category-1 data points.

The difference between the purposeful age bias of –1 and –2 was not resolved. The resolution of this bomb ^{14}C age validation study was limited by the approximate nature of the expected 1-year shift, variability in ^{14}C due to geographic location, and variability in ^{14}C measurement, but the general accuracy of the ages was validated. In using bomb $\Delta^{14}C$ for age validation, assumption 1 is the largest contributor to any concerns about resolution due to variability in $\Delta^{14}C$ because of depth or geographic area (Nydal, 1993; Kalish, 1995; Andrews et al., 2007).

Our method of separating the specimens into two categories addressed specific objectives. Use of only the clearest specimens to validate the age estimates for category-1 specimens is common practice (Piner and Wischniowski, 2004; Kerr et al., 2005; Kastelle et al., 2008). In a long-lived species such as Dover sole, age determination is difficult (Chilton and Beamish, 1982; Kimura and Anderl, 2005) and subjective interpretations of growth patterns (Fig. 2) must be made. When determining the age of a species on a routine basis, a set of species-specific interpretive rules or "aging criteria" are applied to all specimens, i.e., to specimens that are easy to interpret and to those that are difficult to interpret (Kastelle et al., 2008). By choosing validation samples like category 1 where there is little ambiguity, the basic methods and aging criteria can be validated and then applied to all samples, including ones like category 2. If the specimens were chosen randomly, without consideration for the difficulty in estimating age or interpreting otoliths, the spread of validation sample points around the reference chronology would increase. This spread would provide less informative results regarding the basic aging criteria, as shown by the spread in the category-2 specimens. The types of specimens represented by category 2 are more common in Dover sole; hence this sample design was selected to provide the most informative results. The exact percentage represented by the two categories was not determined in this study. A further reason for using different categories was to provide a tool to develop aging criteria for the hard-to-age specimens. Following the validation, the less common category-1 specimens were used to provide the loess fit in Figure 5 to which the category-2 specimens were compared.

The $\Delta^{14}C$ results displayed a gap in specimens at a birth year of 1961. Similar gaps have been seen in other studies (Kerr et al., 2004; Piner and Wischniowski, 2004; Piner et al., 2005; Kastelle et al., 2008) and are likely due to two reasons. First, in the early- to mid-1960s, marine ^{14}C was likely increasing so quickly that even if otoliths were accurately aged, a gap would likely be present because of the limited time range when these mid-range values of $\Delta^{14}C$ existed. Second, in our sample selection process, otoliths that could be categorized unambiguously as category 1 and aged such that they represented the 1961 birth year were not present.

The utility of $\Delta^{14}C$ standardization is apparent in this study. Without the standardization, most of the validation sample $\Delta^{14}C$ points before about 1968 are above the reference chronology, or to its left, and below the reference chronology after 1969. With the $\Delta^{14}C$ standardization and the purposeful bias of –2 years applied to the category-1 ages, it is clear that the Dover sole $\Delta^{14}C$ points and Pacific halibut reference chronology are in synchrony and that ages are accurate. As previously explained, the shift to the left is due to the core size. The consistently low Dover sole $\Delta^{14}C$ points after 1969 are

likely due to different environmental regimes or biological differences of the two species (Kalish, 1993, 1995; Nydal, 1993; Andrews et al., 2007). The standardization procedure is ideal for correcting this type of bias, where a difference in range of $\Delta^{14}C$ exists. Previously, this procedure was used by Kastelle et al. (2008) to re-analyze validation data for black drum (*Pogonias cromis*), originally presented by Campana and Jones (1998). For the re-analysis, the black drum $\Delta^{14}C$ values were standardized to a Northern Hemisphere atmospheric $\Delta^{14}C$ reference chronology in a comparison where they were dramatically different in scale but similar in timing (Kastelle et al., 2008). If little difference in range exists when the standardization is applied, the estimated values of μ and σ will be close to 0 and 1, respectively, provided the overall fit is good. This situation would indicate that the standardization had little effect and that the correct evaluation of any aging error will still be made by considering the SSRs. This was the case for Pacific ocean perch analyzed previously with this method (Kastelle et al., 2008). Therefore, we feel this standardization method can be applied generally.

We chose the Pacific halibut reference chronology for several reasons. First, this reference chronology is based mostly on juvenile fish (Piner and Wischniowski, 2004). It also represents a wide geographic area in the GOA, similar to that for Dover sole. Finally, although the early life history of Dover sole in the GOA is not well understood, the pelagic larvae are found in the upper 30 m of the water column, and immature fish are known to concentrate in nearshore areas and shallow waters over the continental shelf (Abookire et al., 2001; Abookire and Bailey, 2007). Juvenile Pacific halibut are typically found in shallow nearshore areas (Norcross et al., 1995; Abookire et al., 2001); therefore comparisons with Dover sole for this age validation were reasonable.

The otolith cores from the validation samples were smaller than the measured guide otoliths from 3-year-olds. Some of this difference may be explained by the presence of newly deposited opaque material beyond the third translucent zone in the measured 3-year-olds. In the cored otoliths, this same material was often ground away to expose the third translucent zone, thereby producing a size difference. Also, a few of the cores may have been incorrectly centered during the grinding process, and therefore may have incorporated a little material from beyond the third year. Conversely, too much material could have been removed, down to the second year's growth zone. The latter is more likely the case as evidenced by the small core weights. On the proximal side, the coring process may have inadvertently removed some material belonging to the third year in an effort to remove all material from later years in the region of the sulcus groove. We considered the average age of the material represented by the cores to be approximately 1.5 years. However, if the cores were too small and some material inside the third translucent zone was removed, than the average age of the material may have been closer to 1 year, indicating less of a required shift. The difference was only 0.5 years; hence we considered any error from this consideration to be negligible. Less of a required shift was also indicated if the otoliths were accumulating more mass during their first year than in subsequent years (see *Materials and methods* where linear otolith growth is assumed). As mentioned previously, this age validation method can not resolve either type of error when potentially very small. However, it is probably not coincidental that the purposeful age bias of –1 year for the unstandardized $\Delta^{14}C$ results was the best fit.

Dover sole otoliths often display a transition in growth rate typically seen as a pattern of decreased spacing between presumed annual growth zones. This occurs when the fish is estimated to be about 6- to 8-years-old, with growth zones deposited prior to the transition representing younger and faster growth and post-transition zones representing slower growth (Figs. 1 and 2). An association between the transition timing and maturity has not been documented in Dover sole, but Abookire and Macewicz (2003) reported that 50% maturity occurs at 6.7 years which roughly coincides with the observed transition. They used specimens aged by the same experienced age readers as in this study; hence some level of circularity exists. However, our studies' results lead us to believe that the timing of the transition pattern is likely associated with the onset of maturation. In other species such as orange roughy (*Hoplostethus atlanticus*) a decrease in the annual growth zone width is documented to correspond to the onset of maturity (Francis and Horn, 1997).

Considerations concerning category-2 results

Category-2 $\Delta^{14}C$ results confirm that Dover sole are often a difficult-to-age species. This difficulty was exemplified by the range in possible ages of even the youngest category-2 specimens and was especially evident in the two older category-2 specimens. The CV of 4.21% for these hand-picked specimens, where the majority (38 out of 43) of otoliths were deemed to be clear, although better than the typical CV of 9.64% for Dover sole, was high in comparison to that for many other flatfish species aged at the AFSC (Kimura and Anderl, 2005). The category-2 otoliths are typical of many Dover sole samples aged at the AFSC where subjective decisions are made by necessity in the age reading process.

Results from the five difficult-to-age specimens in category 2 did not indicate consistent over-aging or under-aging. Correct decisions in how to interpret the growth zone patterns were made for specimens 225 and 188, and a reasonably good choice was made in specimen 95 especially when its high age was considered. It is clear that the choice of a mid-range or older age was the most accurate for specimens 95 and 188 when compared to the loess-smoothed curve of category-1 specimens. However, specimens 146 and 317 were demonstrated to be over-aged in the comparison to category-1 specimens. The underlying difficulty in aging and interpreting Dover sole otoliths lies in the framework of splits that can make up a single trans-

lucent zone (Fig. 2). In specimens from category 1, the translucent zones were usually compact and well defined; hence any apparent splits could easily be interpreted and decisions could be made as to how they should be enumerated. In category-2 specimens, the potential annual zones were not well defined, often due to splits that created interpretative options. Splits in a potential annual zone that occur before the transition to slow growth are especially problematic. In hindsight, it was reasonable to conclude that the two specimens (numbers 146 and 317) that diverged from the loess-smoothed category-1 data were over-aged probably because of broad splits. The specimen with the largest discrepancy, number 146, was one where the pattern in the broken-and-burnt cross section could be aged at 16 years (more in line with the loess-smoothed category-1 data), or on a second reading axis could be interpreted as 26 years. Similarly, in Figure 2 an age of 32 years was estimated along an axis closer to the sulcus, but a more correct age estimate of 20 years was chosen from an adjacent reading axis. Some of this discrepancy could be the result of splitting translucent zones. In reality, the five specimens in category 2 are not enough to draw firm conclusions on how these difficult otoliths should be aged. A further study of category-2 type specimens may help to refine the aging criteria for Dover sole otoliths.

The region in the otolith cross section before the transition to slow growth can be an area of splitting or diffuse translucent zones. This is a situation that can lead to over-aging; therefore the reader must exercise care to count only prominent translucent zones. Splitting and diffuse translucent zones are a frequent problem in age reading many species (e.g., Francis and Horn, 1997; Gregg et al., 2006; Hutchinson et al., 2007). The fish in category 1 were correctly aged by counting only the prominent translucent zones preceding the transition to slow growth.

Conclusions

The ages estimated for the GOA Dover sole were validated as accurate based on the easy-to-age otoliths in category 1. When the age bias of –1 or –2 years was applied, the $\Delta^{14}C$ in the validation samples had the same timing as the $\Delta^{14}C$ in the Pacific halibut reference chronology and was consistent with the expected 1-year core-size shift. An age-structured stock assessment model is used for management of the GOA Dover sole commercial fisheries and hence the age data validated here are important for population modeling and setting the total allowable catch (Stockhausen et al., 2005). In the future, analysis of additional difficult-to-age category-2 otoliths may help to further answer questions regarding aging criteria for these specimens. In reality, the lower-than-average between-reader precision for growth zone counts will likely persist, but now we have a high degree of confidence in the accuracy of ages estimated from specimens with clear growth zones.

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Abstract—A 4500-year archaeological record of Pacific cod (*Gadus macrocephalus*) bones from Sanak Island, Alaska, was used to assess the sustainability of the modern fishery and the effects of this fishery on the size of fish caught. Allometric reconstructions of Pacific cod length for eight prehistoric time periods indicated that the current size of the nearshore, commercially fished Pacific cod stocks is statistically unchanged from that of fish caught during 4500 years of subsistence harvesting. This finding indicates that the current Pacific cod fishery that uses selective harvesting technologies is a sustainable commercial fishery. Variation in relative Pacific cod abundances provides further insights into the response of this species to punctuated changes in ocean climate (regime shifts) and indicates that Pacific cod stocks can recover from major environmental perturbations. Such palaeofisheries data can extend the short time-series of fisheries data (<50 yr) that form the basis for fisheries management in the Gulf of Alaska and place current trends within the context of centennial- or millennial-scale patterns.

A 4500-year time series of Pacific cod (*Gadus macrocephalus*) size and abundance: archaeology, oceanic regime shifts, and sustainable fisheries

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Fishing is a size-selective process that tends to remove larger fish and reduce the life span and mean size of individuals, especially in heavily exploited systems (Shin et al., 2005; Trites et al., 2006). Reductions in size-at-age and age-at-maturation have been reported for a number of heavily exploited species of fish (Trippel, 1995; Rochet, 1998; Bianchi et al., 2000) and may cause evolutionary change (Ernande et al., 2004; Hutchings, 2005; Law, 2007). Knowledge of body size is therefore an important metric for fisheries management because it can affect market price and reflects the spawning potential of the fish population (Shin et al., 2005).

Most fisheries management and conservation research has been restricted to interpreting trends and data sets that span just a few decades (Pauly et al., 1998; Worm et al., 2006), and tend not to incorporate the longer-term perspective that can be obtained by including palaeoecological data (Willis and Birks, 2006). Combining palaeoecological data with biological data can provide a significantly longer time series for measuring population and ecosystem health for conservation biology and resource management, and for understanding how current decadal trends fit within the context of centennial- or millennial-scale cycles (Jackson et al., 2001; Braje et al., 2006). Humans have been sampling species and ecosystems for thousands of years and have left rich data sets of both natural ecosystem dynamics and human interactions with animal populations that are preserved in archaeofaunal remains (animal bones found on archaeological sites).

Sanak Island is the center of a small, low-lying archipelago on the edge of the continental shelf in the western Gulf of Alaska (Fig. 1). This island group was a hub of the North Pacific Pacific cod (*Gadus macrocephalus)* fishery from the 1870s to the 1930s (Reedy-Maschner, 2004). Local Aleuts reported that Pacific cod disappeared in commercial quantities in this region between 1942 and 1975 but returned and have supported the modern Pacific cod fishery since the 1975 oceanic regime shift.

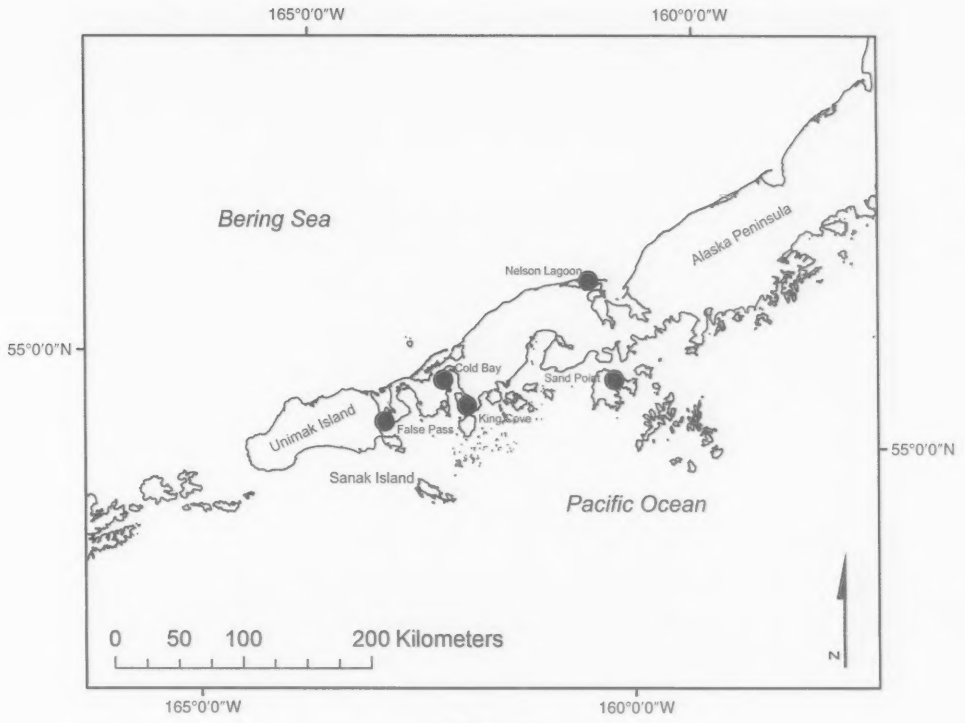

Figure 1

The North Pacific and the western Gulf of Alaska region showing Alaska and the location of Sanak Island where bones were collected at archeological sites to determine whether a change in fish size was evident over the 4500 archeological record.

The Sanak Pacific cod fishery now includes mostly fishermen of Aleut-Scandinavian descent who use jigs, pots, and longlines in nearshore waters, but the fishery has deep, prehistoric roots (Tews, 2005). Archaeological data indicate that Aleuts have occupied Sanak Island for over 6000–7000 years and Pacific cod bones dominate the matrices of all preserved midden deposits from over 120 prehistoric village sites found on the island. These remains provide a novel laboratory for constructing long-term time series of Pacific cod populations.

The goal of this study was to compare archeological data with modern fisheries data to assess whether industrialized fishing has changed the size of Pacific cod in the North Pacific Ocean and to investigate whether or not Pacific cod size and abundance may be influenced by climate change. Pacific cod are among the most heavily exploited and consumed species of fish in the Northern Hemisphere and have declined significantly in most parts of the world in the past 30–40 years. In eastern Canada, fishing resulted in Atlantic cod (*Gadus morhua*) becoming smaller and reaching sexual maturity at younger ages as fisheries reduced the populations by more than 90% (Fanning et al., 2003; Hutchings, 2005; Lilly et al., 2005). Evolutionary reductions in body size caused by fishing have also been reported for Baltic cod (*Gadus morhua callarias*) (Andersen et al., 2007). In the Gulf of Alaska, Pacific cod are generally thought to be fished at sustainable levels, but there is a limited time series to assess whether fisheries have changed the size of fish caught over the past 50 years (Thompson et al., 2006). It is, however, possible to use paleofisheries data to assess whether the lengths and, by correlation, average fecundity of fish caught today are comparable to those caught thousands of years ago before the advent of industrialized fisheries, and to use lengths and frequencies of Pacific cod harvested in prehistory to assess the role of climate in the structure of Pacific cod populations.

Materials and methods

All of the samples used to estimate the sizes of fish over the prehistoric time sequence were derived from shell-midden deposits that were excavated by trowel and sieved through 6-mm mesh, an appropriate sieve size given specimen sizes in the region (e.g., see *Discussion* in Cannon, 1999), and our results were substantiated by direct comparisons with bulk samples processed with 3-mm mesh). In our analysis, we controlled for possible

spatial differences in procurement of samples by including only sites located at the eastern end of the island that were associated with rocky intertidal coastlines. We further controlled for possible seasonal differences in procurement by including only midden deposits that were stratigraphically associated with winter villages (as denoted by the presence of large semisubterranean house ruins). Sample sizes for Pacific cod in each of these faunal assemblages ranged between 48 and 3219 specimens (individual bones). Actual sample sizes for measured Pacific cod skeletal elements ranged from a low of 10 to a high of 507 elements. By archaeological standards, the number of fish bones recovered from these middens was large, and thus the data set was relatively robust. Nevertheless, we recognize the possibility of potential sample-size effects, and present the entire data set as a case study.

The majority of Pacific cod bones recovered contained significant biometric information because they were not fragmented and came from well-preserved deposits. Because length figures prominently in modern fisheries management research (Shin et al., 2005; for the Gulf of Alaska see Thompson et al., 2006), allometric relationships developed by Orchard (2001, 2003) were used to establish the live length of an individual from the skeletal elements of Pacific cod. As an exercise to present the types of data that can be reconstructed from ancient Pacific cod bones, the fecundities of Pacific cod were estimated from their length distributions, by means of regressions developed by Karp (1982). More accurate reconstructions could be based on average fecundity relationships specifically recorded for Pacific cod in the Gulf of Alaska, although these data were not available at the time of publication of this article. Thus, the assemblages of skeletal remains recovered from middens in our study contained information about Pacific cod populations that could be compared to information on modern population structures for a large-scale view of changes in size and abundance over time.

Measurements were taken from the ascending process of premaxillas and the centra of trunk vertebrae from Pacific cod derived from eight archaeological sites spanning the period ca. 2550 BC to 1540 AD (radiocarbon years were calibrated to calendar years by using the methods of Stuiver and Reimer, 1993). Although these data were time averaged, the samples were from single stratigraphic units dated by multiple radiocarbon measurements and likely represent a single depositional episode that took place over one or a few seasons. From these data, the estimated length of the individuals that contributed each element was computed from Orchard's (2001, 2003) regression equations and the combined measures were used to produce length-frequency distributions for each time slice.

The modern length distribution of Pacific cod was obtained from the longline survey done by the Alaska Fisheries Science Center in the Gulf of Alaska in 2005 (n=3308; Thompson et al., 2006). Longline surveys are a fishing method that may be most comparable with prehistoric jigging practices. However, it should be noted that modern longline gear may subtly select for smaller size fish, as discussed by Halliday (2002). The mean longline survey data (Thompson et al., 2006) are presented as frequencies in specific size bins, and mean length was calculated by using procedures in Gedamke and Hoenig (2006).

We also measured the relative abundance of Pacific cod in the prehistoric middens using a measure known as an abundance index (AI). This measure can be used to track shifts in the relative abundance of taxa in relation to other taxa in a faunal assemblage and represents a normed ratio of a highly ranked (in terms of human foraging efficiency) taxa A to a lower ranked taxa B, measured as $AI=A/[A+B]$ (Bayham, 1979; Ugan and Bright, 2001). Values close to 0 indicate a complete absence of taxa A, and values close to 1 indicate a dominating presence. We used an abundance index because they are generally robust and are resistant to taphonomic (conditions affecting preservation) and collection biases, as long as these biases are systemic to all the assemblages being compared (Ugan and Bright, 2001)—a situation applicable to the Sanak Island data. Although simple to calculate, the measures can be powerful. For example, shifts in the index can reflect changes in human foraging efficiency because they incorporate body-size–based caloric relationships. In the model, it is assumed that larger bodied prey are often more highly ranked than smaller bodied prey (because they contain more calories per unit of effort); therefore when a highly ranked or large-bodied prey is compared to a lower-ranked, or smaller-bodied prey, the values can be used to determine the occurrence of resource depressions, or declines in foraging efficiency. Abundance indices are a well-established and peer-reviewed method of measuring changes in the frequencies of taxa through time from archaeological data (Broughton, 1994, 1997; Butler, 2000; Ugan and Bright, 2001; Nagaoka, 2002; Betts and Friesen, 2006).

Problems can arise in the interpretation of AI measures when inappropriate B taxa, or lower-ranked taxa, are chosen for comparison with higher ranked A taxa. Betts and Friesen (2006) demonstrated that these issues can be overcome by 1) carefully selecting B taxa that occur in moderate, but relatively stable frequencies in the assemblages, and 2) comparing multiple low-ranked B taxa and assessing overall trends. Here we compared Pacific cod to smaller-bodied species of fish—Cottidae, Hexagrammidae, Pleuronectidae, and *Oncorhynchus*. The former three taxa were found in the same resource patch (i.e., in the nearshore jig fishery), and likely entered the procurement system as bycatch. The last taxon, salmon, was likely captured at stream mouths and head waters with weirs and nets, and was included in our analyses for comparative purposes. Mean lengths of Pacific cod recovered from different eras were compared by using t-tests and analysis of variance, and linear regressions were used to determine the significance of trends over time.

Table 1

Comparisons of mean fork lengths of Pacific cod (*Gadus macrocephalus*) caught by Aleuts from 1540 AD to 2550 BC compared to the mean fork lengths of fish captured in 2005 by longline surveys in the Gulf of Alaska. Comparisons were made by using *t*-tests, and values in bold indicate no significant difference at the $P<0.01$ level. Sample size *n* is the number of measured bone elements from the prehistoric eras. The site numbers reflect individual winter village sites on Sanak Island from which the archaeological samples were derived.

		Mean fork length (cm)					
Site number	Era	Prehistoric	Modern	*t*-value	df	*P*	*n*
111	2550 BC	67.5	67.5	0.003	26	**0.997**	27
054	1750 BC	70.7	67.5	5.932	573	<0.001	467
036	840 BC	72.1	67.5	5.471	340	<0.001	313
061	595 BC	64.1	67.5	−1.181	9	**0.267**	10
058	80 BC	69.7	67.5	3.801	601	**0.012**	507
056 Upper	520 AD	65.5	67.5	−2.011	175	**0.046**	166
056 Lower	1030 AD	66.6	67.5	−0.981	257	**0.327**	241
110	1540 AD	62.8	67.5	−6.223	515	<0.001	464

Results and discussion

Measuring changes in Pacific cod size and abundance

The mean size of Pacific cod has varied considerably in the Gulf of Alaska over the last 4500 years (Fig. 2; range 62.8–72.1 cm; $F_{(8, 5503)}=11.97$, $P<0.001$). However, modern mean lengths did not differ significantly from the mean lengths of fish caught around 2550 BC, 595 BC, and 1030 AD (Table 1 ; *t*-tests $P>0.05$), or when compared with lengths of those taken around 80 BC and 520 AD (Table 1; *t*-tests $P>0.01$).

Fecundities of Pacific cod (estimated from body length distributions) varied dramatically, ranging between 2.1 and 3.9 million eggs per individual (Fig. 3). Average fecundity varied consistently with changes in mean length as expected, but the apparent trend shown in Figure 3 towards decreasing fecundity over 4500 years was not significant ($r=-0.42$, $P=0.35$).

The AI measures revealed changes in the relative abundance of Pacific cod over the past 4500 years (Fig. 4). All of the AIs were highly variable between 2550 and 2580 BC, but were synchronized after 520 AD. They indicated high relative abundance of Pacific cod in middens spanning 2550–1750 BC and showed an opposite trend in the abundance of salmon, which was consistent with the inverse relationship previously noted for this region and time period (e.g., Tews, 2005; Misarti, 2007). From 840 BC to 80 BC another period of variability occurred when salmon AI was opposite that of the groundfish taxa. During this time, the AIs for Pleuronectidae and Hexagrammidae indicated a slight decline in relative abundance of Pacific cod, whereas the Cottidae index indicated a greater decline in Pacific cod abundance. Taken in tandem, however, all of the groundfish taxa recovered from the

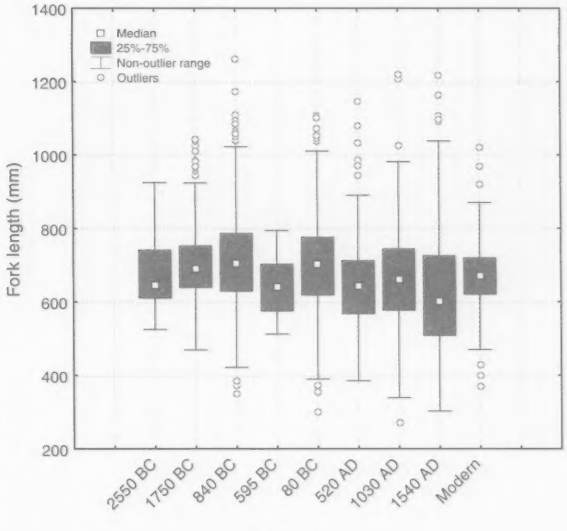

Figure 2

Box plots showing the fork-length (mm) distributions of Pacific cod (*Gadus macrocephalus*) caught in the Gulf of Alaska since 2550 BC. The prehistoric distributions were reconstructed from skeletal elements (premaxilla and centra from trunk vertebrae) by using allometric relationships established by Orchard (2003). The modern length distributions were compiled from data collected from longline surveys conducted in the Gulf of Alaska by the Alaska Fisheries Science Center between 1978 and 2006 (Thompson et al., 2006).

middens indicate that there were slight or moderate declines in Pacific cod during this early period. On the basis of the remains in the middens, there was a sharp decline in the relative abundance of Pacific cod (compared to the other three taxa) beginning around 520 AD. This declining trend intensified drastically in the 1030 AD assemblage, and was followed by a sharp increase in Pacific cod abundance at 1540 AD.

The declining AIs strongly indicate that a form of resource depression occurred from 520 through 1030 AD (for a similar interpretation of declining fish AIs, see Nagaoka, 2001, 2002). Resource depressions can be related to a number of factors, such as exploitation pressure, behavioral changes, and microhabitat (range) shifts (e.g., Charnov et al., 1976). The apparent decrease in Pacific cod abundance that we noted is intriguing given the lack of any technological, procurement, or other cultural changes (e.g., increased territoriality) that could have influenced the encounter or success rate of the Aleut Pacific cod fishery. Instead we suspect the decline in Pacific cod reflects an environmentally-driven natural change in abundance and note that the increase in mean length during this period corroborates this interpretation (see Shin et al., 2005).

Interpreting the effects of climate on Pacific cod size and abundance

Punctuated shifts in ocean climate (regime shifts) are believed to explain many of the changes observed in abundances of some species in the North Pacific Ocean during the past century (Hare and Mantua, 2000; Benson and Trites, 2002; Trites et al., 2007) and these shifts may explain the variations noted in Figure 4. Pacific cod populations may be susceptible to fluctuations in oceanic regimes to the extent that they periodically disappear in significant numbers from the ecosystem, only to reappear in greater numbers at a later date. This condition has deep historical roots; the ancient Aleut name for Pacific cod translates literally into "the fish that stops" because this species periodically disappears (Black, 1981), a situation that occurred, according to traditional Aleut knowledge, at least once in the mid-19th century, and again in approximately 1942.

Several small variations in mean Pacific cod length were evident over the temporal sequence. Correlating the shifts in body size with climatic shifts does not appear to explain the fluctuating sizes of Pacific cod between 2550 BC and 80 BC, which occurred during the generally cool and wet conditions of the Neoglacial period (the first major postglacial cooling period from approximately 2500 BC to AD 1). The average lengths of Pacific cod (Fig. 2) increased as the warming and drying of the Medieval climatic anomaly began (a period of hemispheric climatic fluctuations ca. AD 1000–1300 across the Northern Hemisphere) and decreased slightly during the cool and wet conditions of the Little Ice Age (ca. AD 1400–1850). Marine productivity during the Medieval climatic anomaly and the Little Ice Age, as recorded by Finney et al. (2002) and Misarti (2007), appears to be inversely related to Pacific cod lengths but positively related with Pacific cod numbers (Fig. 4). Such inverse relationships between mean fish length, abundance, and productivity have been noted by Shin et al. (2005) (i.e., increased productivity and abundance is usually associated with decreases in mean length, presumably because of the increase of juvenile fish in the population). The impact of these types of processes on fish lengths over the long time scales has never been assessed, and so this must remain a working hypothesis. Regardless, it is also notable that the mean length of Pacific cod during the Medieval climatic anomaly was not statistically different from that of the modern era codfish (Table 1)—two of the warmest periods in the last 4500 years.

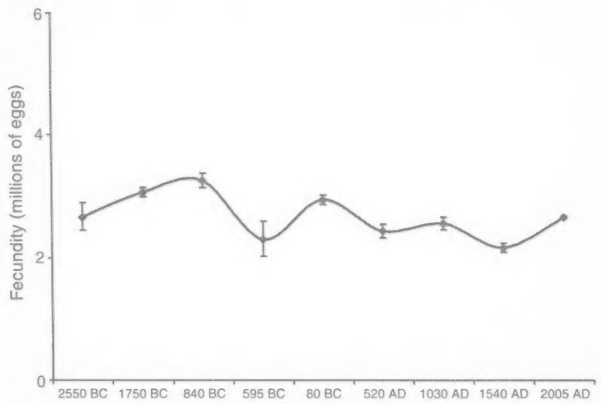

Figure 3

Mean estimated fecundity of Pacific cod (*Gadus macrocephalus*) over time based on the relationships between body length and fecundity established by Karp (1982). This graph indicates that estimated mean fecundity of Pacific cod has varied widely over the past 4500 years, despite concomitantly small changes in mean Pacific cod length (compare with Fig. 2). Although the graph indicates an apparent trend toward decreasing fecundity, the relationship is not significant; r=–0.42, P=0.35.

The effects of oceanic regime shifts that have dominated the dynamics of marine ecosystems in the North Pacific for the past century (Benson and Trites, 2002; Polovina, 2005) are difficult to monitor in the prehistoric period because of an important complicating factor—an overwhelming lack of data. Although climatic and oceanic warming AD 1000 to 1300 have been documented around the North Pacific (Calkin et al., 2001; Hu et al., 2001), and its effects are beginning to be understood (Jones et al., 1999), the full effects of climate change on Pacific cod stocks over the entire period are difficult to measure because not a

single preserved Pacific cod bone dating from AD 1100 to 1300 in the Sanak archaeological data has been recovered despite the presence of village sites. The period between AD 1100 and 1300 were the only centuries during the last 5000 years of extensive global warming that occurred before the modern era. Pacific cod returned in the middens only during the hemispheric cooling associated with the Little Ice Age. Interestingly, regardless of periodic regime shifts in ocean climate, Pacific cod populations appeared to have returned to the western Gulf of Alaska with approximately the same length structure as that before they disappeared.

The perturbations in the body lengths of Pacific cod did not correlate with changes in their frequencies of occurrence in the middens (Fig. 4). For example, the most extreme shift in mean Pacific cod length occurred between 2550 BC and 80 BC, yet Pacific cod abundances varied minimally during this time. It is noteworthy that this entire period occurred during the Neoglacial, a period of generally cooler and wetter oceanic conditions. This relationship may indicate that perturbations in abundance, at least during cold periods, were not related to systemic fluctuations in the length or fecundity structure of the Pacific cod populations in the Gulf of Alaska.

At the end of the temporal sequence ca. 1030 AD and 1540 AD, an inverse relationship is noted between mean length and abundance of Pacific cod. The mean lengths increased during the Medieval climatic anomaly and decreased during the Little Ice Age, whereas overall relative abundances decreased during the Medieval climatic anomaly and increased during the Little Ice Age. This finding would indicate that Pacific cod were more abundant but smaller during cool periods and less abundant but larger during warmer periods. Although the inverse relationship between length and abundance is intriguing, it is uncertain how they are related. The shift in mean Pacific cod length between these periods was small (less than 3 cm), yet abundances fluctuated widely.

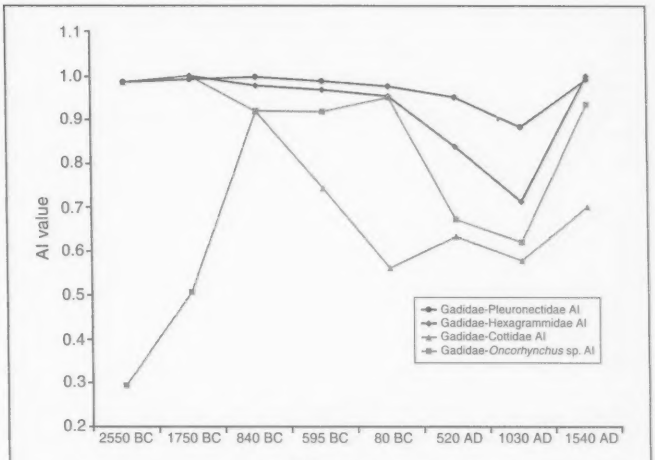

Figure 4

Abundance indices (e.g., *AI=Pacific cod / [comparison taxon + Pacific cod]*), calculated from the number of identified specimens (bone fragments) for each taxon recovered from nine archaeological deposits on Sanak Island. The index ranges between 0 and 1, with 0 indicating the complete absence from the middens and 1 indicating a complete dominance of Pacific cod (*Gadus macrocephalus*). Sample sizes from the deposits ranged between 426 and 8339 identified bone specimens. All indices indicate a decline in Pacific cod abundance beginning at 520 AD, except for the Gadidae-*Oncorhynchus* spp. index, which is included to show the difference between riverine (*Oncorhynchus* spp.) and nearshore marine fish (the other families of fish represented in the graph) during this period. This decline in abundance continues throughout the period until 1030 AD, after which we see a recovery in Gadidae abundance at ca. 1540 AD. The correspondence in the multiple measures negates the mathematical conundrum of closed arrays and indicates real changes in the relative abundance of Pacific cod in Sanak middens.

Climatic regime shifts appear to have had minimum effects on the length structure of Pacific cod populations in the Gulf of Alaska, but have had dramatic effects on their numbers and possibly geographic range. The apparent drop in Pacific cod numbers during periods of warming may reflect a shift in Pacific cod distribution (e.g., Charnov et al., 1976), and may explain why the Aleut word for Pacific cod is the "fish that stops."

General observations regarding the prehistoric Pacific cod data

Additional insights about Pacific cod can be made from the analyzed bones, which show, for example, that Pacific cod have long-term, millennial-scale population dynamics that appear to have a complex relationship with climatic shifts. Pacific cod seem to be vulnerable to major climatic regime shifts, particularly warming conditions, and appear to rapidly recover from major perturbations to their environment. This conclusion would signify that oceanic regime shifts have no long-term (centennial- to millennial-scale) ramifications on the overall structure of Pacific cod populations. Furthermore, Pacific cod stocks appear to have largely maintained their population structure over some 4500 years despite the pressures of commercial harvesting in the modern era.

The trends uncovered for Pacific cod in the North Pacific are significantly different from those in the North Atlantic. Swain et al. (2007) concluded that length distributions can be a key indicator of genetic changes in Pacific cod as a result of overfishing, and that these changes can persist for decades despite low harvest pressure. In particular, they found that rapid evolutionary changes can result from size-selective overfishing, and these changes can far outpace the levels expected for natural mortality as a result of disease or regime shifts.

The archaeological record in the western North Atlantic shows that the average length of Pacific cod caught today by commercial fisheries is at least 40 cm smaller than that of prehistoric times, and indicates that the Pacific cod populations have been impacted by modern fisheries (Kenchington and Kenchington, 1993; Jackson et al., 2001). Similar shifts have been discovered in the central North Atlantic where Amorosi et al. (1994) demonstrated significant differences in the length distributions of Pacific cod between the Medieval period and the modern era. In contrast, we found that the minimum difference in the average length of Pacific cod taken by longline fisheries was only 3–4 cm larger than the smallest prehistoric mean size fish caught by the ancient Aleuts using jigs. The implications of these regional differences are intriguing given the different historical trajectories in commercialization of the groundfish fisheries in these two regions, namely a different emphasis on harvesting technologies (i.e., a much higher proportion of the North Pacific catch is taken with cod pots, compared to the North Atlantic, where cod pots have seen minimal use). The North Pacific cod pot fishery, in particular, reduces the catch of juvenile fish.

This study points to ways in which ancient archaeological deposits can be used to significantly extend the time-depth of fish population studies and can provide important insights into long-term sustainability. Such palaeofisheries research is important for placing modern decadal population trends within a longer-term perspective. In the case of Pacific cod, the paleoecological records indicate that today's Pacific cod are comparable in size to fish that inhabited the Gulf of Alaska thousands of years ago. This finding indicates that modern fisheries have not altered the average length distributions of Pacific cod in the Gulf of Alaska when measured over long time scales.

The fact that modern Pacific cod length distributions are within the range of precommercial variability indicates that the current commercial Pacific cod fishery has sustained the length structure (and therefore average fecundity structure) of the prehistoric (noncommercial) Gulf of Alaska Pacific cod population. Compared to the North Atlantic, the changes we observed in fish length between the prehistoric and modern eras in the Gulf of Alaska are more consistent with natural fluctuations than with harvesting pressure. The fact that the present-day size-structure of Gulf of Alaska Pacific cod stocks is consistent with a sustainable fishery (Thompson et al., 2006) and that the current size-structure is comparable to that of pre-industrially fished populations indicates that current Pacific cod fisheries management policies and harvesting techniques in the western Gulf of Alaska are working. That is, management practices and harvests are maintaining a natural Pacific cod population structure based on mean size as a comparative metric of sustainability. However, we caution that global warming may become a complicating factor for modern management practices given that Pacific cod populations may be vulnerable to pressures caused by increasing oceanic temperatures, as appear to have occurred circa AD 1000–1300.

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Prevalence of the commensal barnacle *Xenobalanus globicipitis* on cetacean species in the eastern tropical Pacific Ocean, and a review of global occurrence

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Abstract—Distribution and prevalence of the phoretic barnacle *Xenobalanus* on cetacean species are reported for 22 cetaceans in the eastern tropical Pacific Ocean (21 million km^2). Four cetacean species are newly reported hosts for *Xenobalanus*: Bryde's whale (*Balaenoptera edeni*), long-beaked common dolphin (*Delphinus capensis*), humpback whale (*Megaptera novaeangliae*), and spinner dolphin (*Stenella longirostris*). Sightings of *Xenobalanus* in pelagic waters are reported for the first time, and concentrations were located within three productive zones: near the Baja California peninsula, the Costa Rica Dome and waters extending west along the 10°N Thermocline Ridge, and near Peru and the Galapagos Archipelago. Greatest prevalence was observed on blue whales (*Balaenoptera musculus*) indicating that slow swim speeds are not necessary for effective barnacle settlement. Overall, prevalence and prevalence per sighting were generally lower than previously reported. The number of barnacles present on an individual whale was greatest for killer whales, indicating that *Xenobalanus* larvae may be patchily distributed. The broad geographic distribution and large number of cetacean hosts, indicate an extremely cosmopolitan distribution. A better understanding of the biology of *Xenobalanus* is needed before this species can be used as a biological tag.

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Barnacles of the superfamily Coronuloidea live as obligate commensals on sea turtles, cetaceans, sirenians, sea snakes, and crustaceans (Newman and Ross, 1976). The monotypic *Xenobalanus globicipitis* Steenstrup, 1851 (herein referred to by genus) is specialized for living as a commensal on whales and dolphins (Darwin, 1854). The typical six-plate balanomorph shell is small and is imbedded into the skin of the cetacean host. The membrane supporting the operculum is greatly elongated, so that externally *Xenobalanus* resembles a pedunculate barnacle. This species is most commonly observed on the trailing edges of the dorsal fin, pectoral flippers, and tail fluke of the host, although it has been reported in areas such as the rostrum and the area between the teeth (Samaras, 1989). *Xenobalanus* does not receive nutrition from its cetacean host and therefore is not considered a parasite. Instead, as a suspension-feeding cirriped, it uses the water flow around swimming cetaceans and benefits from being transported by its host (phoresis). This species is highly specialized to live on cetaceans (Seilacher, 2005) and it has been suggested that its hermaphroditic reproduction may be synchronized with that of its host (Dollfus, 1968; Fertl, 2002). A five- to six-month reoccurrence cycle has been reported for *Xenobalanus* (Van Waerebeek et al., 1993; Orams and Schuetze, 1998), which may indicate that its life span may be of similar length or that occurrence is correlated with seasonal environmental conditions. *Xenobalanus* has been reported on 30 cetacean species worldwide and has a prevalence ranging from 0.5% to 55% of individuals in each sighting. However, intensity is highly variable, and there are some reports of greater than 100 barnacles on a single host (Aznar et al., 2005).

We examined the presence of *Xenobalanus* on cetaceans in the eastern tropical Pacific Ocean (ETP). Based on photographs taken during research cruises from 1977 through 2003, mean prevalence, mean intensity, and geographic distribution are described for *Xenobalanus* on 22 host cetacean species. In addition, peer-reviewed literature on this subject is examined, updating a previous summary of the cetacean hosts of this barnacle (Rajaguru and Shantha, 1992).

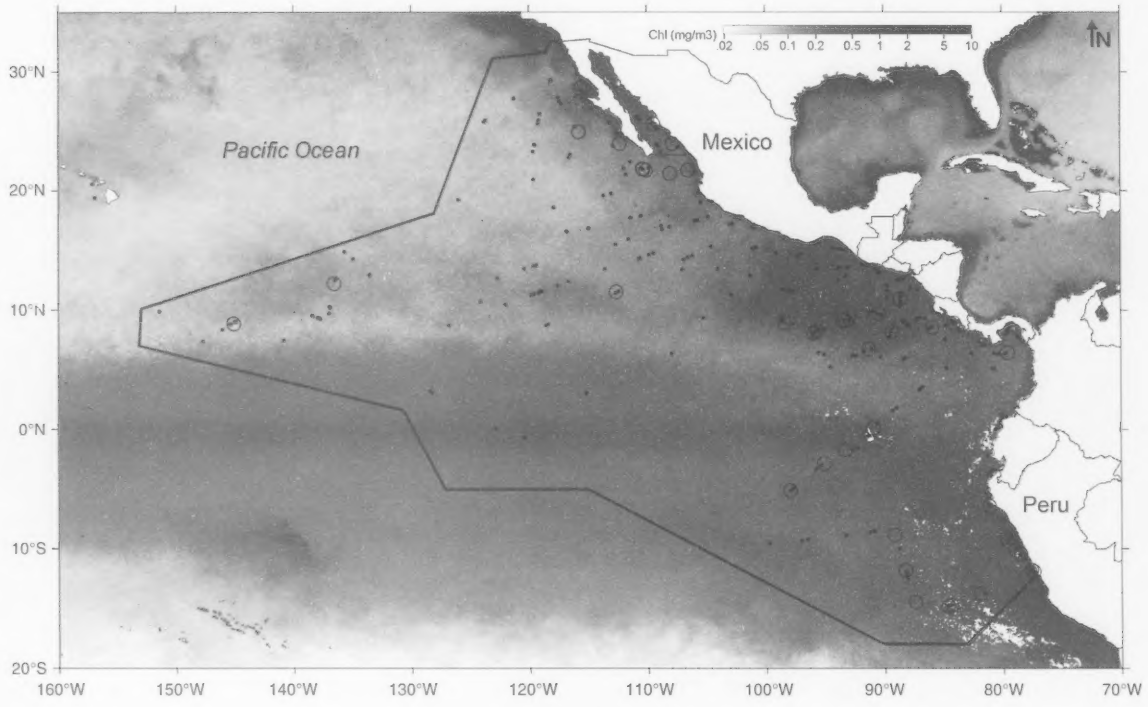

Figure 1

Xenobalanus presence or absence for 445 cetacean sightings in the eastern tropical Pacific Ocean (ETP) in 2003 as determined from analysis of identification photographs. Dots (●) indicate cetacean sightings with no *Xenobalanus* observed; circles (○) indicate sightings with one or more barnacles observed; the solid line indicates the border of the ETP study area. Presence or absence is overlaid on a background of graded shading representing the volume of chlorophyll-*a* (mg/m^3) averaged from September to November 2003.

Materials and methods

Data collection

Cetaceans were photographed during a 2-ship, 4-month research cruise in 2003, covering 26,000 km of transects surveyed for marine mammals (boundaries shown in Figs. 1 and 2). Camera equipment included Canon EOS 10D and D60 digital cameras (Canon USA, Lake Success, NY) with 75–300 mm image-stabilized zoom and 400-mm fixed lenses. Date, latitude and longitude, cetacean species (as identified by trained cruise personnel), and unique sighting number per cetacean group were recorded with each photograph. In the laboratory, additional data were recorded upon examination of photographs, including the number of usable photographs in the sighting (as described below), number of individual cetaceans identified in the sighting, number of individuals infested with *Xenobalanus*, and number of *Xenobalanus* present. If barnacles were clumped in such a way as to compromise the accuracy of the count, the maximum number of discernible barnacles was recorded. The resolution of the digital photographs was such that in most cases, individual barnacles were easily identified.

In external appearance, *Xenobalanus* may be confused with the parasitic copepod *Pennella balaenoptera* (Evans, 1994) or the stalked barnacle *Conchoderma virgatum* (Ruppert et al., 2004). However, the much larger *P. balaenoptera* usually occurs along the flanks, whereas *Xenobalanus* is generally found along the trailing edges of the dorsal fin, pectoral flippers, and the fluke, as has been described for stranded and live cetaceans. Similar to *P. balaenoptera*, *C. virgatum* requires a less specific position for attachment, requiring any hard substrate (such as another barnacle, a tooth, or exposed bone), and *C. virgatum* is considerably lighter in coloration than *Xenobalanus*. Digital photographic quality was sufficient for accurate identification of the commensal *Xenobalanus*; no specimens were obtained for direct examination.

During the cruise, typically many photographs were taken for each sighting. For our study, usable photographs had 1) to be in focus, 2) to be of sufficient resolution to identify a barnacle if present, and 3) to include at least one cetacean dorsal fin. For large schools, only one photograph per school was used in order to prevent recounting individuals. For small schools, only photographs of animals identifiable as individuals, either from field notes or from unique markings or pigmen-

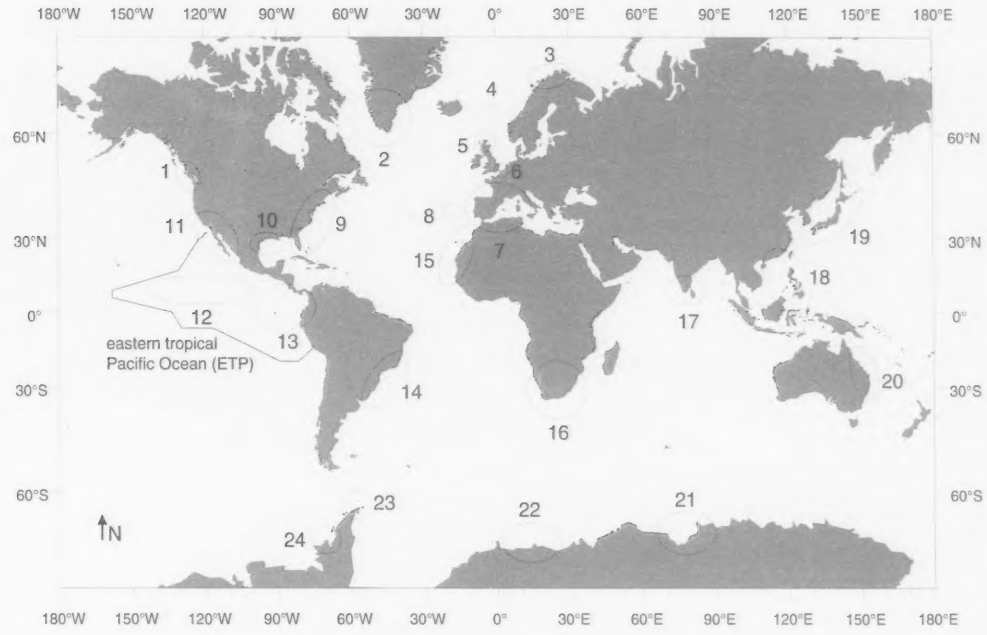

Figure 2

Documented sightings of *Xenobalanus* worldwide on various cetacean hosts compiled from literature review, and an outline of the current study area (eastern tropical Pacific Ocean). Ovals indicate the geographic region where barnacles have been reported and their size does not indicate intensity of infestation. Refer to Table 3 for the corresponding citations for each region.

tation, were used. Photographs used represent a subsample of individuals and did not include every animal observed. Killer whales (*Orcinus orca*) were studied in additional detail, by using photographs taken on previous cruises dating back to 1977, and including photographs from the ETP in the California and Mexico killer whale catalog (Black et al., 1997).

Data analysis

Two measures of prevalence of *Xenobalanus* were calculated for each cetacean species with at least three sightings with usable photographs. Prevalence was calculated for each sighting by dividing the number of individual whales or dolphins with barnacles by the total number of individual cetaceans identified. Mean prevalence and its standard error for each species were calculated from these values. Prevalence per sighting was calculated for each species by dividing the number of sightings with barnacles present by the total number of sightings. Mean barnacle intensity was calculated as the total number of barnacles observed on a host species divided by the number of infested hosts of the same species (Bush et al., 1997). To relate barnacle presence to primary productivity, the presence or absence of *Xenobalanus* at each cetacean sighting in 2003 was plotted on a map of average surface chlorophyll concentration in the ETP that was based on data provided by the SeaWiFS Project, NASA/Goddard Space Flight Center, and GeoEye.

Several statistical tests were performed to determine significant differences among the data. A nonparametric Mann Whitney-*U* test was used to determine if the rates of prevalence differed between Mysticetes and Odontocetes and a nonparametric Kruskal-Wallace test was used to determine if the prevalence rates differed significantly among species. Chi-square goodness-of-fit test was used to determine if the number of barnacles per killer whale followed a Poisson distribution. After normalization, linear regression was used to determine if the number of barnacles observed was predicted by the total number of animals observed in the sighting.

Results

Within the ETP over 10,000 photographs of 22 cetacean species and 2510 individuals revealed that 132 individuals of 14 species were host to *Xenobalanus* (Table 1). Out of 497 photographed sightings, 445 were determined to be usable in the analysis, and of these sightings, 47 displayed *Xenobalanus*: 38 odontocete sightings and 9 mysticete sightings. *Xenobalanus* was not observed on seven cetacean species and on one genus for which the species could not be identified: pygmy killer whale

Table 1

Summary of photographic data collected for cetaceans in the eastern tropical Pacific Ocean from 1977 through 2003, including the number of usable photographs ("photographs"), number of sightings ("sightings"), number of identifiable individual cetaceans ("individuals"), and number of identifiable individuals infested with *Xenobalanus* ("infested individuals"). ♦ Denotes newly reported hosts of *Xenobalanus* that were determined from this study.

Species	Common name	Years	Photographs	Sightings	Individuals	Infested individuals
Balaenoptera edeni♦	Bryde's whale	2003	415	30	43	3
Balaenoptera musculus	blue whale	2003	513	17	24	9
Balaenoptera physalus	fin whale	2003	92	4	6	1
Delphinus capensis♦	long-beaked common dolphin	2003	228	13	69	2
Delphinus delphis	short-beaked common dolphin	2003	1146	48	287	3
Feresa attenuata	pygmy killer whale	2003	25	1	11	0
Globicephala macrorhynchus	short-finned pilot whale	2003	1551	34	297	9
Grampus griseus	Risso's dolphin	2003	155	12	58	1
Lagenodelphis hosei	Fraser's dolphin	2003	34	1	6	0
Lagenorhynchus obliquidens	Pacific white-sided dolphin	2003	5	1	2	0
Lagenorhynchus obscurus	dusky dolphin	2003	245	11	68	4
Megaptera novaeangliae♦	humpback whale	2003	504	12	34	1
Mesoplodon spp.	unidentified *Mesoplodon*	2003	40	2	3	0
Orcinus orca	killer whale	1977–2003	1160	49	354	69
Peponocephala electra	melon-headed whale	2003	17	1	9	0
Physeter macrocephalus	sperm whale	2003	215	9	19	0
Pseudorca crassidens	false killer whale	2003	49	2	11	0
Stenella attenuata	pantropical spotted dolphin	2003	1281	76	326	4
Stenella coeruleoalba	striped dolphin	2003	845	51	319	18
Stenella longirostris♦	spinner dolphin	2003	770	39	271	3
Steno bredanensis	rough-toothed dolphin	2003	118	12	41	0
Tursiops truncatus	bottlenose dolphin	2003	600	61	252	5
Total			10,008	486	2510	132

(*Feresa attenuata*); Fraser's dolphin (*Lagenodelphis hosei*); Pacific white-sided dolphin (*Lagenorhynchus obliquidens*); melon-headed whale (*Peponocephala electra*); sperm whale (*Physeter macrocephalus*); false killer whale (*Pseudorca crassidens*), rough-toothed dolphin (*Steno bredanensis*); and three unidentified beaked whale individuals (*Mesoplodon* spp.) Of these, the barnacle has been previously reported throughout its worldwide range on pygmy killer whale, false killer whale, two species of beaked whales (*Mesoplodon* spp., Rajaguru and Shantha, 1992), Pacific white-sided dolphin (Dailey and Walker, 1978), and rough-toothed dolphin (Addink and Smeenk, 2001).

Four cetacean species seen in the ETP had not previously been reported as hosts of *Xenobalanus*: Bryde's whale (*Balaenoptera edeni*), long-beaked common dolphin (*Delphinus capensis*), humpback whale (*Megaptera novaeangliae*), and three forms of spinner dolphin (*Stenella longirostris*): eastern (*S. longirostris orientalis*) and the forms known commonly as whitebelly and southwestern spinner dolphins (Table 2). For Bryde's whales and humpback whales, the dorsal fin was the only visible appendage, as opposed to the long-beaked common dolphins and spinner dolphins for which pectoral flippers and tail flukes were also visible. The humpback whale individual that displayed a single specimen of *Xenobalanus* appeared to have a damaged dorsal fin.

Prevalence and intensity of the barnacle

Blue whales (*Balaenoptera musculus*) had the highest mean prevalence of the barnacle, followed by fin whales (*B. physalus*) and killer whales (Fig. 3A). There was a significant difference in mean prevalence among species (χ^2_{30}=50.6, P<0.01) and Mysticetes had a higher mean prevalence of the barnacle than Odontocetes (5.1% vs. 0.8%). Standard error was greatest for blue (13.8) and fin whales (16.5); all other species had a standard error less than 1.5. Blue, fin, and killer whales also had the highest prevalence per sighting, and 38% of killer whale sightings had barnacles (Fig. 3B). Prevalence per sighting was similar for Mysticetes and Odontocetes (12.7% vs. 14.0%)—a nonsignificant difference (P=0.19). Of the three species most often infested, killer whales repre-

Table 2

Dates and geographic locations (latitude and longitude) for sightings of newly documented cetacean hosts of *Xenobalanus* in the eastern tropical Pacific Ocean in 2003. Data are the following: total number of individuals of the species photographed ("individuals"), total number of these individuals observed with barnacles ("infested individuals"), and barnacle intensity and anatomical location on the host ("intensity and location on the host").

Host	Individuals	Infested individuals	Intensity and location on the host	Date	Geographic location
Balaenoptera edeni Bryde's whale	64	3	4 on dorsal fin	03 November 2003	09.012°S 079.302°W
Delphinus capensis long-beaked common dolphin	69	1	1 on right pectoral flipper	12 August 2003	25.620°N 109.456°W
Megaptera novaeangliae humpback whale	34	1	1 on dorsal fin	05 November 2003	06.414°S 081.176°W
Stenella longirostris orientalis eastern spinner dolphin	99	1	1 on left pectoral flipper	15 August 2003	21.448°N 108.084°W
Stenella longirostris hybrid whitebelly spinner dolphin	91	1	1 on dorsal fin	20 August 2003	08.857°N 145.098°W
Stenella longirostris southwestern southwestern spinner dolphin	32	1	2 or more on right pectoral flipper	14 October 2003	05.084°S 097.974°W

sented the majority of individuals used in the analysis (14%), whereas blue whales (1%) and fin whales (0.2%) were rarely encountered. The number of barnacles was, therefore, independent of number of individuals observed (R^2=0.00, F=0.10, P=0.08).

Xenobalanus was found in coastal as well as offshore waters of the ETP (Fig. 1). All 22 species were represented in offshore sightings and 28% of individuals encountered were seen in waters greater than 600 km from land, and at a maximum distance of 4287 km from land. Of these offshore occurrences, 39 *Xenobalanus* were observed on 18 individuals comprising seven species. *Xenobalanus* was primarily observed in three areas: 1) waters around the Baja California peninsula, 2) the Costa Rica Dome and waters extending west along the 10°N Thermocline Ridge, and 3) waters off Peru and the Galapagos Archipelago. All three areas are known as areas of increased primary productivity within the ETP (Fig. 1, Fiedler et al., 1991; Pennington et al., 2006).

For killer whales, which were examined in more detail, of the 68 whales infested with 130 barnacles, the mean intensity of infestation was 1.9 barnacles per whale. The greatest numbers of killer whales were photographed in 1998–2003, and these whales also had the greatest intensity of barnacles. This observed increase in intensity was most likely the result of improved photographic techniques. The observed numbers of killer whales with 0, 1, 2, 3, and >3 barnacles were 286, 38, 15, 7, and 8, respectively. This is significantly different from the expected 245, 90, 17, 2, and 0 infested whales, respectively, as predicted by a Poisson distribution with mean 130/354 = 0.367 (χ^2_5>300, P<0.00001). The variance (0.941) was much larger than the mean (0.367).

Literature review

A chart of the worldwide distribution of *Xenobalanus* was generated from a review of the literature documenting regional occurrences of this genus (Fig. 2). Except for the ETP, *Xenobalanus* has been reported only within approximately 600 km from land, including the Faröe Islands and the Azores (sites 4 and 8). Figure 2 also demonstrates that *Xenobalanus* is highly cosmopolitan and has been reported in all oceans, namely in tropical, temperate, and polar waters.

The literature review updates a previous review conducted by Rajaguru and Shantha (1992). Eighteen peer-reviewed accounts have been published since that review, including that of the present study (Table 3). Additionally, ten records of *Xenobalanus* had not been included in Rajaguru and Shantha's (1992) review. An additional 14 cetacean species are now included: minke whale (*Balaenoptera acutorostrata*), Bryde's whale, long-beaked common dolphin, Pacific white-sided dolphin, dusky dolphin (*Lagenorhynchus obscurus*), right whale dolphin (*Lissodelphis borealis*), humpback whale, vaquita (*Phocoena sinus*), Burmeister's porpoise (*Phocoena spinipinnis*), franciscana (*Pontoporia blainvillei*), clymene dolphin (*Stenella clymene*), spinner dolphin,

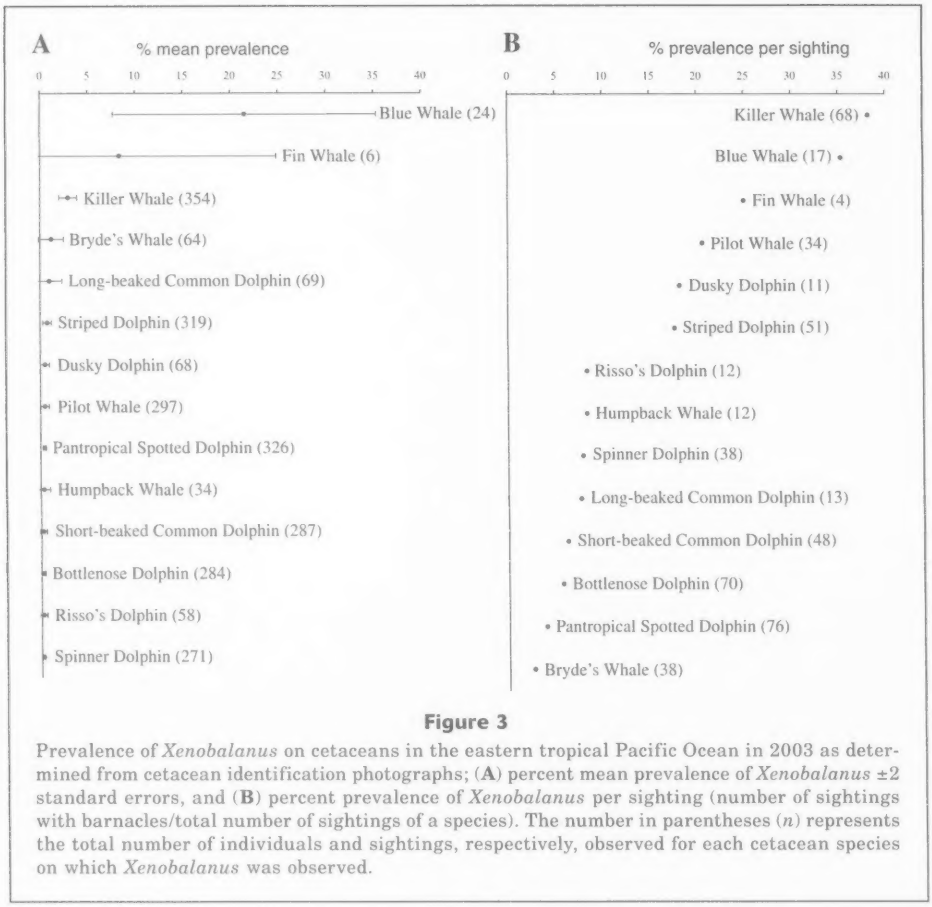

Figure 3

Prevalence of *Xenobalanus* on cetaceans in the eastern tropical Pacific Ocean in 2003 as determined from cetacean identification photographs; (**A**) percent mean prevalence of *Xenobalanus* ±2 standard errors, and (**B**) percent prevalence of *Xenobalanus* per sighting (number of sightings with barnacles/total number of sightings of a species). The number in parentheses (*n*) represents the total number of individuals and sightings, respectively, observed for each cetacean species on which *Xenobalanus* was observed.

rough-toothed dolphin, and Indo-Pacific bottlenose dolphin (*Tursiops aduncus*).

Discussion

Prevalence and intensity of the barnacle

We describe four new host species for the cetacean-specific phoretic barnacle *Xenobalanus* and document that the barnacle is present on cetaceans far offshore as well as in coastal areas. The fact that *Xenobalanus* has now been reported on 34 species of cetaceans in both coastal and offshore waters, from the Arctic to Antarctic, either 1) indicates that the barnacle is extremely cosmopolitan (Newman and Ross, 1976; Spivey, 1981), or 2) may suggest that more than one species of the genus *Xenobalanus* is involved.

Mean prevalence results from this study were lower than those from previous published accounts: 0.2% vs. 4–19% for short-beaked common dolphins (*Delphinus delphis*) (Pilleri, 1970; Dailey and Walker, 1978), 0.2% vs. 43–56% for bottlenose dolphins (*Tursiops truncatus*) (Di Beneditto and Ramos, 2004; Toth-Brown and Hohn, 2007), and 0.7% vs. 33–43% for striped dolphins (*Stenella coeruleoalba*) (Pilleri, 1970; Aznar et al., 2005). Two striped dolphins have been reported with an intensity of more than 100 *Xenobalanus* (Aznar et al., 2005), but the greatest intensity observed in our study was seven, on killer whales.

Although some differences in prevalence are due to previous reports of maximum, rather than mean, rates, the prevalence of *Xenobalanus* infestation reported in this study is underestimated because not all barnacles present on the animals were visible in our photographs. Only one side of the animal was photographed, and often part of the body was in the water. On the other hand, prevalence reported in many previous studies may have been overestimated when rates were based on mortality events and strandings. Because stranded animals are not usually healthy, the reported rates could represent an abnormal presence of the barnacle, as was observed in Aznar et al. (2005). Differences may also be related to habitat. Moreover, previous reports have

Table 3

Geographic regions and corresponding citations for each region where *Xenobalanus* has been documented on cetaceans worldwide. "Circle" refers to the regions encircled in Figure 2. References cited in Rajaguru and Shantha (1992) have been omitted individually, but are included under the citation for Rajaguru and Shantha (1992). Additional data about new hosts determined in the present study are available in Table 2.

Circle	Geographic region	Citation
1	Pacific Northwest United States and Canada	Rajaguru and Shantha (1992)
2	Greenland	Rajaguru and Shantha (1992)
3	Northern Scandanavian peninsula	Rajaguru and Shantha (1992)
4	Feröe Islands	Rajaguru and Shantha (1992)
5	Scotland and Shetland Islands	Rajaguru and Shantha (1992)
6	Belgium	Rajaguru and Shantha (1992)
7	Western Mediterranean and Iberian Peninsula	Raga and Carbonell (1985), Rajaguru and Shantha (1992), Aguilar and Raga (1993), Resendes et al. (2002), Aznar et al. (2005)
8	Azores	Rajaguru and Shantha (1992)
9	East coast United States and the Bahamas	Rajaguru and Shantha (1992), Toth-Brown and Hohn (2007)
10	Gulf of Mexico	Spivey (1981), Jefferson et al. (1995)
11	Southern California and Baja Peninsula	Dailey and Walker (1978), Brownell et al. (1987), Samaras (1989), This study
12	Pelagic ETP	This study
13	Costa Rica Dome, Galapagos, and Peru	Van Waerebeek et al. (1990, 1993), Reyes and Van Waerebeek (1995), Palacios et al. (2004), This study
14	Southeast coast of Brazil and Uruguay	Brownell (1975), Young (1991), Rajaguru and Shantha (1992), Di Beneditto and Ramos (2000, 2001, 2004)
15	Northwestern coast of Africa	Van Bree (1971), Rajaguru and Shantha (1992), Addink and Smeenk (2001)
16	South Africa and Namibia	Rajaguru and Shantha (1992)
17	Southern India	Rajaguru and Shantha (1992), Karuppiah et al. (2004)
18	Philippines, South China Sea, Hong Kong	Parsons et al. (2001)
19	Japan	Uchida and Jun (2000), Sakai et al. (2006)
20	East coast of Australia	Rajaguru and Shantha (1992), Orams and Schuetze (1998)
21	Mawson and Davis seas, Antarctica	Bushev (1990)
22	Riiser-Larsen and Lazarev Seas, Antarctica	Bushev (1990)
23	Shetland Islands, northwest Weddell Sea, Antarctica	Bushev (1990)
24	Bellinghausen Sea, Antarctica	Bushev (1990)

primarily been composed of data from coastal areas, whereas our study was focused on pelagic waters.

Factors affecting the presence of *Xenobalanus*

Other behavioral and environmental factors may also affect barnacle presence on cetaceans within the ETP. Swimming speed of the host has been shown to correlate negatively with intensity of the whale lice *Isocyamus delphini* (Balbuena and Raga, 1989) and has been hypothesized as an inversely proportional factor in *Xenobalanus* settlement (Orams and Schuetze, 1998; Aznar et al., 2005). In our study, blue whales had the greatest mean intensity of *Xenobalanus* and have been shown to sustain cruising speeds up to 33 km/hr (Yochem and Leatherwood, 1985), indicating that swimming speed may not be a primary factor in host species selection for *Xenobalanus*. Abrasive breaching and slapping behavior of the host may scour barnacles and inhibit settlement; however, some barnacles appear resistant (Felix et al., 2006; Sakai et al., 2006). In the ETP, deep-diving sperm whales (*Physeter macrocephalus*) and beaked whales (*Mesoplodon* spp.) were not hosts, indicating that dive depth of the host may limit the settlement of the barnacle on these species. Orams and Schuetze (1998) and Toth-Brown and Hohn (2007) have suggested an environmen-

tal correlation in the distribution of *Xenobalanus* that is similar to that observed between primary production and barnacle presence in the ETP. Plankton abundance in oligotrophic areas of the ETP may be below a critical threshold for the filter-feeding barnacles and may thus indirectly limit the presence of *Xenobalanus*.

The intensity of barnacles on killer whales in the ETP was not randomly distributed. There were more whales with no barnacles and with three or more barnacles than would be expected if barnacles settled randomly on killer whales, indicating that if *Xenobalanus* larvae settle, it is most often in groups of three or more. This aggregated or contagious distribution could occur as a result of: 1) a chemical cue emitted from the host that induces settlement (Nogata and Matsumura, 2005), 2) a chemical cue emitted from conspecifics that induces settlement (Knight-Jones, 1953), which was suggested for *Xenobalanus* by Aznar et al. (2005), 3) patchily distributed barnacle larvae, or 4) an inability of the host to slough newly settled larvae (Ridgway et al., 1997). The low variance in prevalence and the nonuniform distribution of *Xenobalanus* sightings within the ETP indicate that most species are equally selected and that barnacle recruitment may be the result of patchily distributed larvae.

Xenobalanus has been reported on a wide variety of cetacean hosts, and this apparent lack of specialization could provide insight into evolutionary age of *Xenobalanus*. Various species of cyamid whale lice are highly specialized for a particular species of right whale (*Eubalaena* spp., Kaliszewska et al., 2005). *Xenobalanus* is more of a generalist than whale lice, given its apparent ability to settle on various cetacean hosts, which may indicate that its evolution and relationship with cetaceans may be more recent than that of other cetacean commensals, and that its specialization to host species has not yet occurred. Coronulid whale barnacles did not appear in the fossil record until approximately 23 million years ago (Newman and Ross, 1976; Seilacher, 2005), after the appearance of Mysticetes and Odontocetes in the fossil record approximately 35 million years ago. However, it is unknown at what point the genus *Xenobalanus* arose, and presently no data exist on the evolutionary age of cyamids for comparison. In the ETP, *Xenobalanus,* appearing on almost every cetacean species encountered, did not exhibit the degree of host specialization observed in whale lice. With a lack of data on evolutionary age, these findings support only the hypothesis that *Xenobalanus* is a generalist cetacean barnacle.

Biological tags

The relationship between commensals and their hosts, which can indicate host movement and host distributional patterns, is often used to make inferences into the biology and ecology of the host. Comparison of internal parasite fauna has helped distinguish stocks, determine stock associations, track large-scale movements, and identify new recruits to populations in many species of fish, elasmobranchs, invertebrates, and marine mammals (Williams et al., 1992). Among marine mammals, intestinal parasites, whale lice, and barnacles have proven useful for tracking migrations of gray whales (*Eschrichtius robustus*; Killingley, 1980) and identifying stocks and the social structure of pilot whales (*Globicephala melas*; Balbuena and Raga, 1993), and have been useful for tracking general movement patterns of wide ranging, elusive cetacean populations without the use of expensive tagging equipment.

Our results indicate that *Xenobalanus*, however, would not be useful as a biological tag. Within the ETP, *Xenobalanus* is widely distributed and a single, definitive source or home range was not determined for this species. However, this is the first study where distribution of *Xenobalanus* has been systematically examined on a large scale and it is possible that the few offshore observations within the ETP are not representative of global distribution. Although *Xenobalanus* could not be used as a biological tag to track the movements of cetaceans within the ETP in this study, the potential use of *Xenobalanus* as a biological tag should not be abandoned completely. Increased knowledge of the biology of the barnacle, such as host-selection criteria, environmental tolerance limits, and early life history strategies could provide a finer resolution of the phoretic relationship with cetacean species that would enable the use of *Xenobalanus* as a biological tag in future studies. This and other research on *Xenobalanus* will form a useful part of the study of cetacean biology and ecology.

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Onshore-offshore distribution and abundance of tuna larvae (Pisces: Scombridae: Thunnini) in near-reef waters of the Coral Sea

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Abstract—The on-offshore distributions of tuna larvae in near-reef waters of the Coral Sea, near Lizard Island (14°30′S, 145°27′E), Australia, were investigated during four cruises from November 1984 to February 1985 to test the hypothesis that larvae of these oceanic fishes are found in highest abundance near coral reefs. Oblique bongo net tows were made in five on-offshore blocks in the Coral Sea, ranging from 0–18.5 km offshore of the outer reefs of the Great Barrier Reef, as well as inside the Great Barrier Reef Lagoon. The smallest individuals (<3.2 mm SL) of the genus *Thunnus* could not be identified to species, and are referred to as *Thunnus* spp. We found species-specific distributional patterns. *Thunnus* spp. and *T. alalunga* (albacore) larvae were most abundant (up to 68 larvae/100 m^2) in near-reef (0–5.5 km offshore) waters, whereas *Katsuwonus pelamis* (skipjack tuna) larvae increased in abundance in the offshore direction (up to 228 larvae/100 m^2, 11.1–18.5 km offshore). Larvae of *T. albacares* (yellowfin tuna) and *Euthynnus affinis* (kawakawa) were relatively rare throughout the study region, and the patterns of their distributions were inconclusive. Few larvae of any tuna species were found in the lagoon. Size-frequency distributions revealed a greater proportion of small larvae inshore compared to offshore for *K. pelamis* and *T. albacares*. The absence of significant differences in size-frequency distributions for other species and during the other cruises was most likely due to the low numbers of larvae. Larval distributions probably resulted from a combination of patterns of spawning and vertical distribution, combined with wind-driven onshore advection and downwelling on the seaward side of the outer reefs.

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Large-scale (100s km) distributions of tuna larvae (family Scombridae), particularly of the commercially important genera *Thunnus* and *Katsuwonus*, have been extensively investigated because of the need to identify spawning locations and the possibility of estimating spawning stock biomass from surveys of larvae (Strasburg, 1960; Richards, 1976; Scott et al., 1993). Despite this effort, and the apparent abundance and fecundity of *T. albacares* (yellowfin tuna) and *K. pelamis* (skipjack tuna) in the western and central Pacific Ocean, relatively low mean concentrations (2–4 larvae/100 m^3, Leis et al., 1991) and abundances of tuna larvae (2–8 larvae/10 m^2, Leis et al., 1991) have been found during most sampling programs. Low numbers of tuna larvae in any one site or region may simply reflect a broadly distributed, but sparse, spawning pattern of adults, as indicated by the wide geographical ranges of *T. albacares* and *K. pelamis* larvae. Other possible explanations, however, are that previous sampling scales of 100s km between samples were too coarse to account for spatial variability in abundances (Davis et al., 1990a), and that tuna larvae are more abundant in undersampled near-reef areas (Leis et al., 1991).

Nearly all sampling of tuna larvae has been conducted in the open ocean because of the oceanic distributions of adults; however relatively high concentrations of tuna larvae have been found in previously undersampled tropical near-reef (<5 km offshore) locations in three studies (Miller, 1979; Leis et al., 1991; Boehlert and Mundy, 1994). Concentrations of larval *T. albacares* were up to two orders of magnitude greater within 2 km of leeward (west) Oahu Island, Hawaii, than published concentrations in surrounding oceanic waters (Miller, 1979), and *Thunnus* spp. and *K. pelamis* larvae were up to 100 times more concentrated within 200 m of coral reefs in French Polynesia than in oceanic waters of the central Pacific Ocean (Leis et al., 1991). Because adjacent oceanic locations were not simultaneously sampled in these near-reef studies, a direct comparison of these two habitats was not possible. Furthermore, comparisons between the near-reef values of larval tuna concentration determined by Miller (1979) and Leis et al. (1991), and those determined for oceanic habitats in the central Pacific Ocean in other studies, may also be confounded because of differences in sampling methods employed between the two

habitats. Near-reef concentrations were determined by using short (<15 min.) horizontal tows at or near the surface (Miller, 1979; Leis et al., 1991), whereas oceanic sampling in the region has generally involved tows of longer duration (≥30 min.), with nets towed either horizontally at the surface or obliquely from depths reaching 200 m (Matsumoto, 1958; Nakamura and Matsumoto, 1966). Because distributions of tuna larvae are vertically stratified, and *Thunnus* spp. larvae are rarely found at depths greater than 50 m (Davis et al., 1990b; Boehlert and Mundy, 1994), oblique tows to depths in excess of 100 m most likely underestimate concentrations in shallower strata. Because of this situation, it is appropriate to use the measure of abundance (i.e., the number of larvae below a standard surface area of water) rather than concentration (numbers of larvae per standard volume) to compare numbers of larvae among sites where tows were taken to different depths.

Abundances of tuna larvae were determined at both near-reef (2 km offshore) and oceanic sites (~30 km offshore) around the Hawaiian island of Oahu by Boehlert and Mundy (1994) by oblique sampling to 200 m depth. The abundance of *Thunnus* spp. larvae decreased with increasing distance offshore, but only on the leeward side of Oahu Island. Interestingly, the abundance of *K. pelamis* larvae actually increased with distance from the reef on both sides of the island (Boehlert and Mundy, 1994), indicating the possibility of important taxa-specific differences in near-reef distributions of tuna larvae.

The possibility that tuna larvae are generally concentrated near islands and reefs, and not in the open ocean, has important implications for the protection of these vulnerable life stages. Large numbers of tuna larvae near shore have been found in only two regions in the central Pacific Ocean, and only near oceanic islands; therefore further investigation of the generality of this phenomenon is required. Considering that patches of highly concentrated (10,945 larvae/500 m^3) *T. maccoyii* (southern bluefin tuna) larvae in the North East Indian Ocean were only 5–15 km in diameter (Davis et al., 1990a), further investigation of fine-scale patterns of larval tuna distribution is also warranted.

The aim of the present study was to investigate the near-reef abundance and on-offshore distributions of tuna larvae in the Coral Sea, near the Great Barrier Reef, Australia, over a fine (1–10 km) scale. Although the sampling design was originally intended for investigation of distributions of reef fish larvae (Leis, 1986; Leis and Reader, 1991), the sampling scale and gradation of habitat from near-reef to oceanic in the offshore direction also made it appropriate for investigating the near-reef distributions of tuna larvae.

Materials and methods

Study area and experimental design

Larval fish samples were collected in an area of the Coral Sea between Lizard Island (14°30′S, 145°27′E) and 19 km seaward of the outer ribbon reefs of the Great Barrier Reef, Australia (Fig. 1). Lizard Island is situated approximately halfway across the Great Barrier Reef Lagoon (hereafter "lagoon") where water depths range from 25 to 40 m. The outer reefs lie on the continental shelf break, beyond which depth increases rapidly reaching 2000 m within 12 km. There is an abrupt change from shallow, protected waters of the lagoon to oceanic conditions in the offshore direction. Winds were usually from the E to SE during the study period (common for this region); therefore the near-reef waters that we sampled were on the windward side of the outer reefs.

Four cruises were conducted to investigate the horizontal distribution of fish larvae: 1) 2–5 November 1984 (early November cruise), 2) 17 and 20–22 November 1984 (late November cruise), 3) 30 January–2 February 1985 (early February cruise), and 4) 9–13 February 1985 (late February cruise). On each cruise, six samples were taken in each of five on-offshore blocks defined by distance (nautical miles, nmi) from the outer reef crest: A) 0–0.25 nmi (0–0.46 km), B) 0.25–1.0 nmi (0.46–1.85 km), C) 1.0–3.0 nmi (1.85–5.56 km), D) 3.0–6.0 nmi (5.56–11.1 km), and E) 6.0–10.0 nmi (11.1–18.5 km, Fig. 1). On each cruise, six samples were also taken in the lagoon between Lizard Island and the outer reefs (Fig. 1). Samples were taken over four consecutive days on each cruise. Offshore transects were planned to be conducted over three days, and two samples to be taken in each block at randomly chosen distances from the reef on each day. All samples in the lagoon were taken on the same day. Transects were centered on a different reef each day and were started from opposite ends on alternate days. Distance from the reef was determined by radar reflection off the waves breaking on the outer reef crest. Because of this method of measurement, actual distance from the reef varied by approximately 100 m depending on the tide and sea state. Because of bad weather on the second cruise, two days elapsed between the sampling of lagoon and offshore waters, and only two samples could be taken in block A. Offshore transects were conducted over four days during the fourth cruise because of mechanical problems.

Sampling procedure

Quantitative, double-oblique plankton tows were made from a 14-m catamaran with a bongo net (cylinder-cone mesh design) with 0.85 m mouth diameter and 0.5-mm mesh. The net was towed at approximately 1 m/s and was fitted with both a calibrated mechanical flowmeter and a calibrated mechanical depth and distance recorder. Tows usually filtered 1000–2000 m^3 of water with a mean volume (and standard deviation) of 1554 (585), 1644 (629), 1637 (306), and 1348 (278) m^3 for each cruise, respectively. All tows were completed during daylight, between one hour after sunrise and one hour before sunset. Tows were taken to a target depth of 200 m on the first cruise and to 120 m thereafter, except in the lagoon and block A where they were taken as close to the bottom as considered safe. The net hit bottom

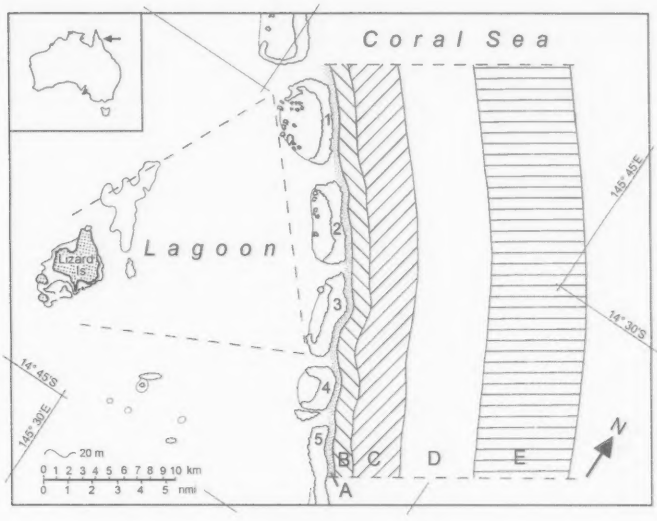

Figure 1

Map of the study area in the Great Barrier Reef Lagoon and Coral Sea, near Lizard Island, Australia. Lagoon samples were taken between Lizard Island and the outer barrier reefs, within the boundaries indicated by dashed lines. Offshore samples were taken in five blocks, A–E, in the Coral Sea. The outer reefs are 1) Day, 2) Carter, 3) Yonge, 4) No Name, and 5) Number 10 Ribbon. Map adapted from Leis et al. (1987).

occasionally in block A because of great variation in water depth in this area. Samples were fixed in formalin (5–10% in seawater) in the field.

Laboratory procedure

Larvae from both the port and starboard sides of the bongo net were sorted, except for those in the lagoon samples from the first and second cruises where the catch from only one randomly chosen side was sorted because of high plankton volumes. Samples from block D were not sorted because of funding cuts to the research program. Larvae were removed with the aid of a dissecting microscope and transferred to 70% ethanol for storage. Tuna larvae (family Scombridae) were identified to species, when possible, by using the descriptions of Fritzsche (1978) and Nishikawa and Rimmer (1987). Larvae of *T. albacares* and *T. alalunga* (albacore) <3.2 mm standard length (SL) could not be separated and were identified as *Thunnus* spp. larvae. For larger larvae, Richards et al. (1990) advocate also using an osteological character, rather than relying solely on pigment, when identifying *Thunnus* larvae to species. However, their study was primarily concerned with *T. atlanticus* (blackfin tuna), a species that is not found in our study area. Further, the osteological character in *T. alalunga* and *T. albacares* seems to vary about as much as does the pigment character, and the former cannot be used in specimens <6 mm SL (Richards et al., 1990). Therefore, we relied on the pigment character to separate our *T. alalunga* and *T. albacares* larvae ≥3.2 mm SL. We note, however, that one-third of the *Thunnus* larvae >6 mm SL that we cleared and stained had pigment inconsistent with the osteological characters listed in Table 1 of Richards et al. (1990). Therefore, according to the criteria of Richards et al. (1990, Table 1), at least 67% of our *Thunnus* larvae are correctly identified to species, and between 0 and 33% may be misidentified to species. Given the variability in osteological features noted by Richards et al. (1990, their Tables 2 and 3), we cannot be more precise about this "uncertain" 33%. Larvae of the genus *Auxis* cannot currently be identified to species (Nishikawa and Rimmer, 1987), and adults of both *A. thazard* (frigate tuna) and *A. rochei* (bullet tuna) inhabit the region of the study area (Collette and Nauen, 1983). *Auxis* larvae were therefore all identified as *Auxis* spp. Larvae of *Auxis* spp. and *Euthynnus affinis* (kawakawa) <2.3 mm SL could not be separated and were identified as *Auxis-Euthynnus* larvae. Notochord length and SL were measured to the nearest 0.1 mm for preflexion and postflexion larvae, respectively, by using a calibrated ocular micrometer. No correction was made for shrinkage of the larvae.

Statistical analyses

Abundances (no. of larvae/100 m^2) were calculated by 1) calculating larval concentration (larvae/m^3), 2) multiplying concentration by the depth (m) sampled, and 3) multiplying the result by 100 to obtain appro-

Table 1

Species composition of tuna larvae (family Scombridae) caught during four cruises in the Coral Sea, near the Great Barrier Reef, between November 1984 and February 1985. Values are numbers of larvae caught. Taxa are ordered to allow comparison of larval numbers among groups comprising the same genera.

Taxon	Early November cruise	Late November cruise	Early February cruise	Late February cruise	Species total	Proportion of total catch (%)
Katsuwonus pelamis	55	89	109	94	347	34
Thunnus spp.	3	24	116	29	172	17
Thunnus albacares	11	19	49	16	95	9
Thunnus alalunga	69	62	26	14	171	17
Auxis-Euthynnus	13	2	72	9	96	10
Euthynnus affinis	49	4	54	1	108	11
Auxis spp.	5	0	10	7	22	2
Total	205	200	436	170	1011	

priately scaled values. Abundance values incorporate a depth component, and are therefore appropriate for comparing oblique samples taken down to different depths. Statistical analyses of on-offshore patterns of distribution of tuna larvae were performed on natural log-transformed abundances, following inspection of the data for normality and heterogeneity of variance. A count of 1 was added to all data-points before transformation, to allow transformation of zero values. Preflexion and postflexion larvae were combined for on-offshore analyses. For each taxon, analysis was done only for cruises with an average larval abundance >1 larvae/100 m^2 to avoid problems associated with numerous zeros. Lagoon samples were not included in calculation of the cruise average because few larvae of any tuna taxon were caught in the lagoon. Only *K. pelamis* larvae were sufficiently abundant for analysis on all four cruises, and their abundance among on-offshore blocks (including the lagoon) and cruises was compared by using a two-factor analysis of variance (ANOVA). For other taxa, abundance among blocks was analyzed with a one-factor ANOVA for each cruise with larval abundance >1 larvae/100m^2. When ANOVA tests yielded significant ($P<0.05$) results, pairwise differences between blocks were analyzed using Tukey's test. *Auxis* spp. larvae were not sufficiently abundant on any cruise to allow for statistical analysis.

Size-frequency data from blocks A and B (inshore zone, 0–1.85 km from the reef) were pooled and compared with size-frequency data pooled from blocks C and E (offshore zone, >1.85 km from the reef) using the Kolmogorov-Smirnov (K-S) test. Data were pooled to increase *n*, because few taxa had sufficient numbers of larvae in each block to provide adequate statistical power. K-S tests were done on data from individual cruises. Larvae from the lagoon were excluded from size-frequency analysis because of low abundance. The significance level used for all statistical tests was 0.05. Not enough *E. affinis* larvae were caught in both zones on any cruise to allow a statistical comparison of size distributions.

Results

Species composition and abundance

Over 1000 tuna larvae were caught, comprising at least five species and four genera (Table 1). Larvae of *K. pelamis* were the most abundant, making up over one third of all tuna larvae caught. Numerous small (<3.2 mm SL) *Thunnus* spp. larvae were caught on the early February cruise, coinciding with a peak in abundance of less common *T. albacares* larvae. In contrast, *T. alalunga* larvae were most abundant on the November cruises, and only 26 individuals were caught on the early February cruise. It is, therefore, likely that most of the *Thunnus* spp. larvae were *T. albacares*. Larvae of other *Thunnus* species (e.g., *T. obesus* [bigeye tuna] or *T. tonggol* [longtail tuna]) were not caught and would have been distinguishable from *T. albacares* and *T. alalunga*, even at small (<3.2 mm SL) sizes (Fritzsche, 1978). *Euthynnus affinis* larvae were most common on the early November and early February cruises, and *Auxis-Euthynnus* larvae were most abundant on the early February cruise. Only 22 *Auxis* spp. larvae were caught during the study, therefore *Auxis-Euthynnus* larvae were most likely primarily *E. affinis* larvae.

On-offshore distribution

Tuna larvae generally had near-reef distributions, as greatest abundances usually occurred within 5.6 km of the outer reefs of the Great Barrier Reef in the Coral Sea. Patterns of on-offshore distribution differed among taxa, however.

Species of *Thunnus* had the most consistent near-reef distribution among the taxa of tuna larvae, with

highest abundances found within 5.6 km of the outer reefs on most cruises with sufficient numbers of larvae for statistical analysis. *Thunnus* spp. larvae were more abundant in block C than in either the lagoon or block E on the late November cruise (Tukey's test, $P<0.01$, Fig. 2) (blocks A and B being intermediate), and more abundant in blocks A and B than in the three other blocks, which did not differ, on the early February cruise (Tukey's test, $P<0.03$, Fig. 2). *Thunnus* spp. larvae showed a similar inshore distribution on the late February cruise; however the only significant difference was a greater abundance of larvae in block B than in the lagoon (Tukey's Test, $P<0.03$, Fig. 2). *Thunnus alalunga* larvae were more abundant in blocks B and C than in the lagoon on the early November cruise (Tukey's test, $P<0.02$, Fig. 3) (the the other blocks being intermediate) and more abundant in block A than in either the lagoon or block E on the early February cruise (Tukey's test, $P<0.04$, Fig. 3). A similar pattern of abundance of *T. alalunga* larvae occurred on the late November cruise; however differences among blocks were not quite significant (Tukey's test, $P=0.051$, Fig. 3). *Thunnus albacares* larvae were most abundant in block A on the early February cruise; however there were no significant differences among blocks (ANOVA, $P=0.06$, Fig. 4).

Distributions of *E. affinis* larvae and *Auxis-Euthynnus* larvae were similar to that of *T. alalunga*, and appeared to be most abundant within 5.6 km of the outer reefs. Differences in larval abundance among blocks were not, however, significant for the early November (ANOVA, $P=0.14$, Fig. 5) and early February (ANOVA $P=0.11$, Fig. 5) cruises analyzed for *E. affinis*, or the early February (ANOVA, $P=0.06$, Fig. 5) cruise analyzed for *Auxis-Euthynnus*.

In contrast to other tuna taxa, the abundance of *K. pelamis* larvae increased in the offshore direction. Despite a significant interaction among blocks and cruises (ANOVA, $P=0.004$), *K. pelamis* larvae were more abundant in block E than in blocks A, B, and the lagoon on the early November cruise (Tukey's test, $P<0.02$, Fig. 6), more abundant in blocks C and E than in the lagoon on the early February cruise (Tukey's test, $P<0.002$, Fig. 6), and more abundant in block E than the lagoon on the late February cruise (Tukey's test, $P<0.05$, Fig. 6). The statistical interaction among blocks and cruises was most likely caused by a relatively great abundance (mean 12.1 larvae/100 m^2) of *K. pelamis* larvae in the lagoon on the late November cruise, compared with abundance in the lagoon on the early November, early February, and late February cruises (means 0.6, 0.0, and 3.5 larvae/100 m^2, respectively, Fig. 6). Apart from *K. pelamis* and *T. alalunga* larvae on the late November cruise, few larvae of any tuna taxon were found in the lagoon.

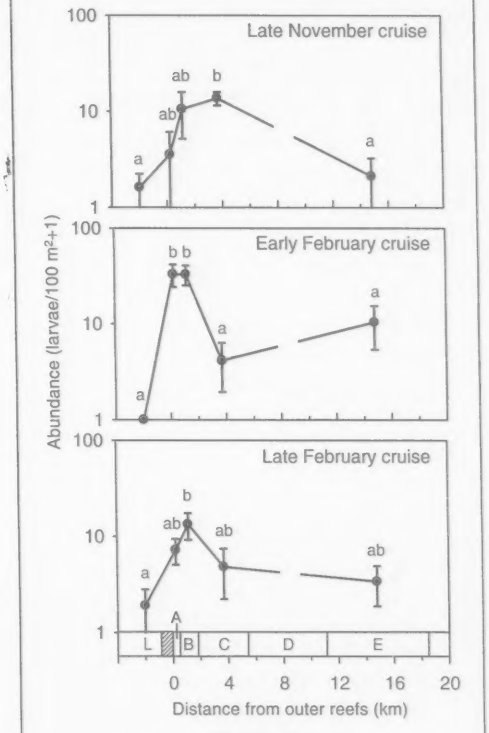

Figure 2

Mean abundance (larvae/100 m^2+1 [± standard error]) of *Thunnus* spp. larvae with distance from the outer reefs of the Great Barrier Reef during the late November, early February, and late February cruises in the Coral Sea. Data points indicate the midpoint of sampling blocks (A–E) by distance, except for the Great Barrier Reef Lagoon (L on the x axis), the display of which is categorical and does not reflect the true distance from the other blocks. The y axis is $\log_{10}$ scale. The x axis shows the width of each sampling block (km). The hatched area on the x axis indicates the position of the outer reef area of the Great Barrier Reef. Within a cruise, if data points share a lowercase letter, they were not significantly different from each other according to Tukey's *post hoc* test. Abundance data was not obtained for block D.

Size-frequency distribution

Tuna larvae ranged in size from 1.7 to 15 mm SL; however most taxa had a strong size mode at 2–4 mm SL. For all species, larvae were of similar size on each cruise, which were at least seven days apart.

Differences in size-frequency distributions of larvae between inshore and offshore zones of the Coral Sea were taxa-specific, however for those tuna taxa where significant differences were detected, there was a greater proportion of small larvae in the inshore zone. A greater proportion of small (2–3.5 mm SL) *K. pelamis* larvae was found in the inshore zone, compared with the offshore zone, on both the early February

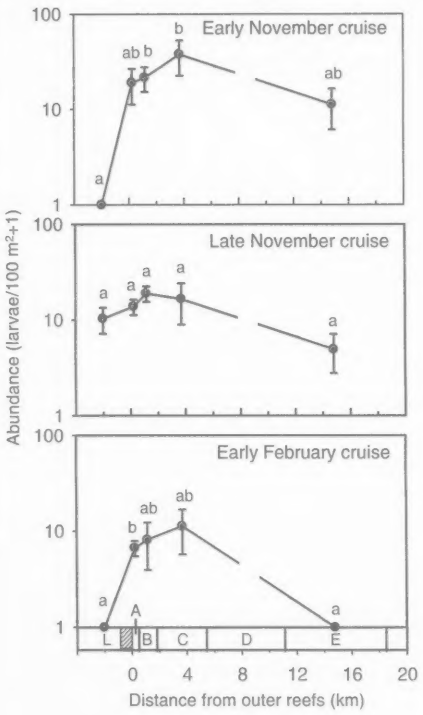

Figure 3

Mean abundance (larvae/100 m^2+1 [± standard error]) of *Thunnus alalunga* (albacore) larvae with distance from the outer reefs of the Great Barrier Reef during the early November, late November, and early February cruises in the Coral Sea. Data points indicate the midpoint of sampling blocks (A–E) by distance, except for the Great Barrier Reef Lagoon (L on the x axis) the display of which is categorical and does not reflect the true distance from the other blocks. The y axis is $\log_{10}$ scale. The x axis shows the width of each sampling block (km). The hatched area on the x axis indicates the position of the outer reef area of the Great Barrier Reef. Within a cruise, if data points share a lowercase letter, they were not significantly different from each other according to Tukey's *post hoc* test. Abundance data were not obtained for block D.

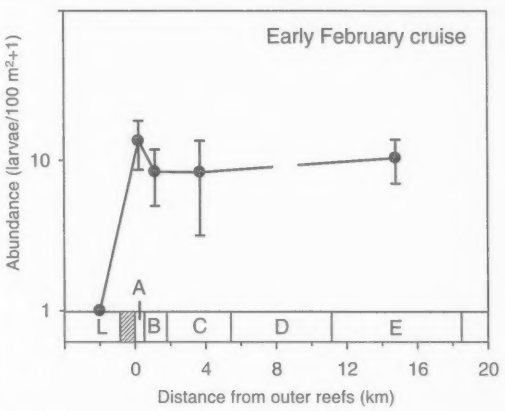

Figure 4

Mean abundance (larvae/100 m^2+1 [± standard error]) of *Thunnus albacares* (yellowfin tuna) larvae with distance from the outer reefs of the Great Barrier Reef during the early February cruise in the Coral Sea. Data points indicate the midpoint of sampling blocks (A–E) by distance, except for the Great Barrier Reef Lagoon (L on the x axis) the display of which is categorical and does not reflect the true distance from the other blocks. The y axis is $\log_{10}$ scale. The x axis shows the width of each sampling block (km). The hatched area on the x-axis indicates the position of the outer reef area of the Great Barrier Reef. No significant differences were found among blocks (ANOVA, P=0.06). Abundance data were not obtained for block D.

(K-S test, P<0.01, Fig. 7A) and late February (K-S test, P<0.02, Fig. 7B) cruises. A similar pattern was found for the late November cruise, with the result approaching significance (K-S test, 0.05<P<0.10, Fig. 7C). Despite a greater proportion of smaller larvae present in the inshore zone, small (~2 mm SL) *K. pelamis* larvae were present in the offshore zone on all cruises. There was also a greater proportion of small (3–4 mm SL) *T. albacares* larvae in the inshore zone, compared with the offshore zone, on the early February cruise (K-S test, P<0.02, Fig. 8). Not enough *T. albacares* larvae were caught in each zone on other cruises for statistical analysis; however examination of size-frequency data indicated that there may also have been a greater proportion of smaller *T. albacares* larvae in the inshore zone on the late November cruise. No significant differences in size-frequency distributions were found for *T. alalunga* larvae between the inshore and offshore zones on either the early November (K-S test, P>0.2, Fig. 9A) or late November (K-S test, P>0.2, Fig. 9B) cruises. There was also no significant difference in the size of *Auxis-Euthynnus* larvae between the inshore and offshore zones on the early February cruise (K-S Test, P>0.2, Fig. 9C).

Discussion

Mean near-reef (<4 km offshore) abundances of tuna larvae in the Coral Sea (range 20–120 larvae/100 m^2) were similar to those found around the Hawaiian island of Oahu (~3–80 larvae/100 m^2, Boehlert and Mundy, 1994) and similar to estimates of near-reef abundance from French Polynesia (45–75 larvae/100 m^2, Leis et al., 1991). Although these values were not much greater

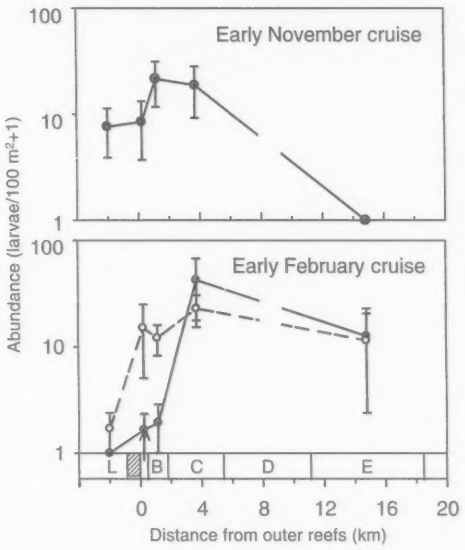

Figure 5

Mean abundance (larvae/100 m^2+1 [± standard error]) of larvae with distance from the outer reefs of the Great Barrier Reef for *Euthynnus affinis* (kawakawa, full line) larvae during the early November and early February cruises, and for *Auxis-Euthynnus* (dashed line) larvae during the early February cruise, in the Coral Sea. Data points indicate the midpoint of sampling blocks (A–E) by distance, except for the Great Barrier Reef Lagoon the display of which (L on the *x* axis) is categorical and does not reflect the true distance from the other blocks. The *y* axis is $\log_{10}$ scale. The *x* axis shows the width of each sampling block (km). The hatched area on the *x* axis indicates the position of the outer reef area of the Great Barrier Reef. No significant differences were found among blocks on either the early November (ANOVA, P=0.14) or early February (ANOVA, P=0.11) cruises for *E. affinis* larvae, or on the early February cruise (ANOVA, P=0.06) for *Auxis-Euthynnus* larvae. *Auxis-Euthynnus* larvae were not sufficiently abundant on the first cruise for statistical analysis. Abundance data were not obtained for block D.

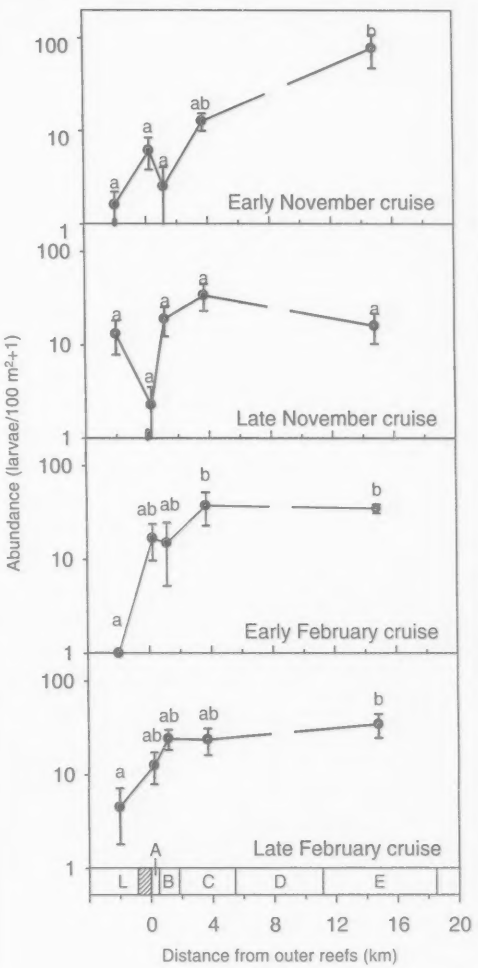

Figure 6

Mean abundance (larvae/100 m^2+1 [± standard error]) of *Katsuwonus pelamis* (skipjack tuna) larvae with distance from the outer reefs of the Great Barrier Reef during four cruises in the Coral Sea. Data points indicate the midpoint of sampling blocks (A–E) by distance, except for the Great Barrier Reef Lagoon the display of which (L on the *x* axis) is categorical and does not reflect the true distance from the other blocks. The *y* axis is $\log_{10}$ scale. The *x* axis shows the width of each sampling block (km). The hatched area on the *x* axis indicates the position of the outer reef area of the Great Barrier Reef. Within a cruise, if data points share a lowercase letter, they were not significantly different from each other according to Tukey's *post hoc* test. Abundance data were not obtained for block D.

than those determined for oceanic sites elsewhere (24–80 larvae/100 m^2, Strasburg, 1960; Nakamura and Matsumoto, 1966), the larvae of most tuna species were more abundant within 5.6 km of the Great Barrier Reef than further offshore in the Coral Sea. This pattern was consistent among cruises for particular taxa, indicating that near-reef larval distributions persist over seasonal time scales.

Larvae of the genus *Thunnus* may generally be more abundant in near-reef waters than farther offshore, because in all studies of tuna larvae in near-reef waters consistently high concentrations or abundances of *Thunnus* larvae have been found there. Greatest abundances of *Thunnus* larvae were often found within 2 km of the outer Great Barrier Reef in the present study,

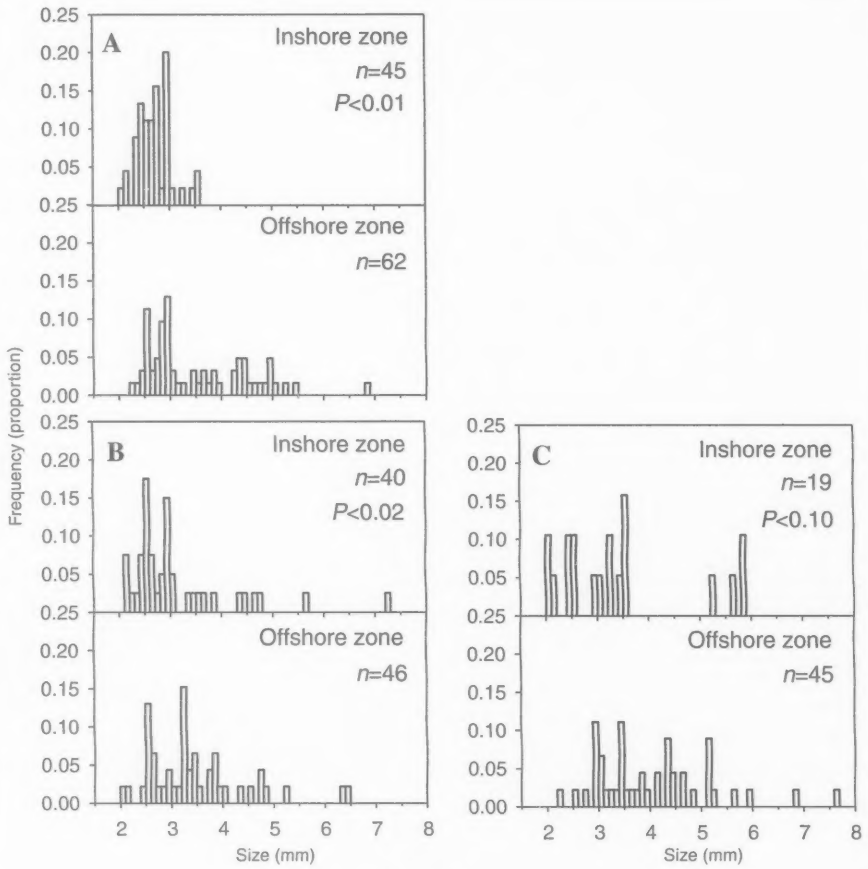

Figure 7

Size-frequency distributions for *Katsuwonus pelamis* (skipjack tuna) larvae from inshore and offshore zones during the (**A**) early February, (**B**) late February, and (**C**) late November cruises in the Coral Sea. Standard length (mm) of larvae was measured to the nearest 0.1 mm. *P*-values refer to the significance of Kolmogorov-Smirnov (K-S) tests conducted between the inshore and offshore zones within a cruise.

and, as with our findings, small (≤3.0 mm SL) *Thunnus* spp. larvae were ~10 times more abundant 1.8 km offshore, than 9.3 km offshore, of the leeward side of Oahu Island (Boehlert and Mundy, 1994). In an earlier study at Oahu Island high concentrations (up to 220 larvae/500 m^3) of *T. albacares* larvae were found within 2 km of shore (Miller, 1979), and similar concentrations (up to 224 larvae/500 m^3) of *Thunnus* spp. larvae (either *T. albacares* or *T. alalunga*) were found in samples taken within 200 m of reefs in French Polynesia (Leis et al., 1991). The present study is the first to investigate near-reef distributions of tuna larvae outside of central Pacific Ocean island environments and thus extends the near-reef distributional pattern to include continental slope environments of the western Pacific Ocean, but further research on on-offshore patterns of abundance in other regions is required to confirm the generality of this phenomenon.

Considerable differences in on-offshore larval distributions may exist among genera of tuna, perhaps even among species, and these differences may not necessarily reflect similarities in adult distributions. We have confirmed the opposing on-offshore distributions of *Thunnus* spp. and *K. pelamis* larvae previously discovered in Hawaii, where *Thunnus* spp. larvae were more abundant near the reef on the leeward side of Oahu Island, while *K. pelamis* larvae increased in abundance in the offshore direction (Boehlert and Mundy, 1994). Interestingly, the on-offshore distributions of *Thunnus* spp. larvae in the Coral Sea were more similar to the larval distributions of *E. affinis* (and possibly *Auxis* spp.) than to the distributions of *K. pelamis* larvae.

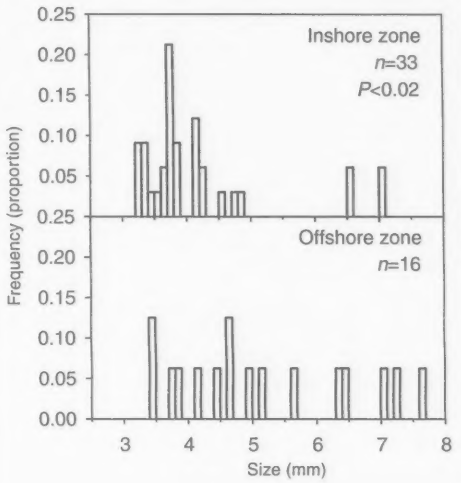

Figure 8

Size-frequency distributions for *Thunnus albacares* (yellowfin tuna) larvae from inshore and offshore zones during the early February cruise in the Coral Sea. Standard length (mm) of larvae was measured to the nearest 0.1 mm. The *P*-value refers to the significance of a Kolmogorov-Smirnov (K-S) test conducted between the inshore and offshore zones.

This finding is remarkable, considering *T. albacares* and *K. pelamis* are considered to be truly oceanic species with similar adult distributions, whereas adult *E. affinis* and *Auxis* spp. have coastal distributions (Collette and Nauen, 1983). Because of potential distributional differences, further research on the near-reef larval distributions of other tuna species is required; however this may be difficult considering the relative rarity of the larvae of some species (e.g., *T. tonggol*).

The greater abundances of small *Thunnus* spp. and *Auxis-Euthynnus* larvae within 5.6 km of the outer Great Barrier Reef indicates that these species may have spawned more intensely or more frequently (or both) in this area, than farther offshore, during the study period. Larvae of *Thunnus* spp. were all <3.2 mm SL because of the limits of our ability to identify small larvae, and therefore their near-reef distribution observed on at least three cruises was most likely the result of near-reef spawning activity of *T. albacares* (which likely comprised most of the *Thunnus* spp. larvae). In support of this conclusion, there was a greater proportion of small *T. albacares* larvae within 1.85 km of the outer Great Barrier Reef than farther offshore during the early February cruise. *Auxis* spp. or *E. affinis* (or both) may have also spawned near the reef in early February, as indicated by the greater abundance of small (<2.3 mm SL) *Auxis-Euthynnus* larvae within 5.6 km of the outer GBR, which approached significance (ANOVA, $P=0.06$, Fig. 5). Their narrow size range (1.9–2.2 mm SL) did not, however, allow for a comparison of sizes between inshore and offshore zones. Although it is likely that initial spawning distributions of the larvae of these two taxa would have been modified to some degree by subsequent physical or biological processes, or both (see below), their small size (and likely young age) would have minimized the time between spawning and capture and therefore would have reduced the potential effect of subsequent modification on their observed distributions.

The greater abundance and size of *K. pelamis* larvae offshore indicates that observed distributions of this species most likely arose from considerable modification of initial spawning distributions. Like *T. albacares*, *K. pelamis* likely spawned more intensely or more frequently, or both, within 1.85 km of the outer Great Barrier Reef during the study period because there was a greater proportion of small larvae within the inshore zone than in the offshore zone, on two, possibly three, cruises. Larval abundance of this species increased with increasing distance from the outer Great Barrier Reef, however, indicating that larvae may have accumulated in the offshore area. A similar pattern of increasing abundance offshore, combined with smaller larvae near the reef, was found for *K. pelamis* on the leeward side of Oahu Island, Hawaii (Boehlert and Mundy, 1994); however no mechanism was suggested to account for these patterns. Differential growth or mortality, or both, for *K. pelamis* larvae may have occurred between near-reef and offshore areas; however we believe that offshore transport by means of physical mechanisms (see below) provides the best explanation of observed distributions, at least in the Coral Sea.

The scarcity of larvae of any tuna taxon in the Great Barrier Reef Lagoon, even when offshore abundances were quite high, indicates that little, if any, spawning occurred there. The moderate abundances of *K. pelamis* (13.1 larvae/100 m^2) and *T. alalunga* (10.3 larvae/100 m^2) larvae in the lagoon on the late November cruise were most likely caused by advection of larvae through the inter-reef passages from spawning locations on the seaward side of the outer reefs, either by onshore winds, or by tidal movement (Leis et al., 1987). We cannot exclude the possibility that some individuals of these species spawned inside the lagoon during the study period, but such spawning would have represented only a small proportion of total spawning effort.

The high abundances of *Thunnus* spp. and *T. alalunga* larvae found near the reef in the present study likely resulted, at least in part, from onshore advection due to wind-driven currents interacting with the surface-orientated distribution of the larvae. The relatively consistent light to moderate onshore (E-SE) winds during the study period in the Coral Sea most likely resulted in shoreward advection of surface water layers. Onshore advection of surface water and subsequent downwelling on the windward side of the outer reefs, combined with a shallow vertical distribution of larvae, was suggested as a possible mechanism resulting in

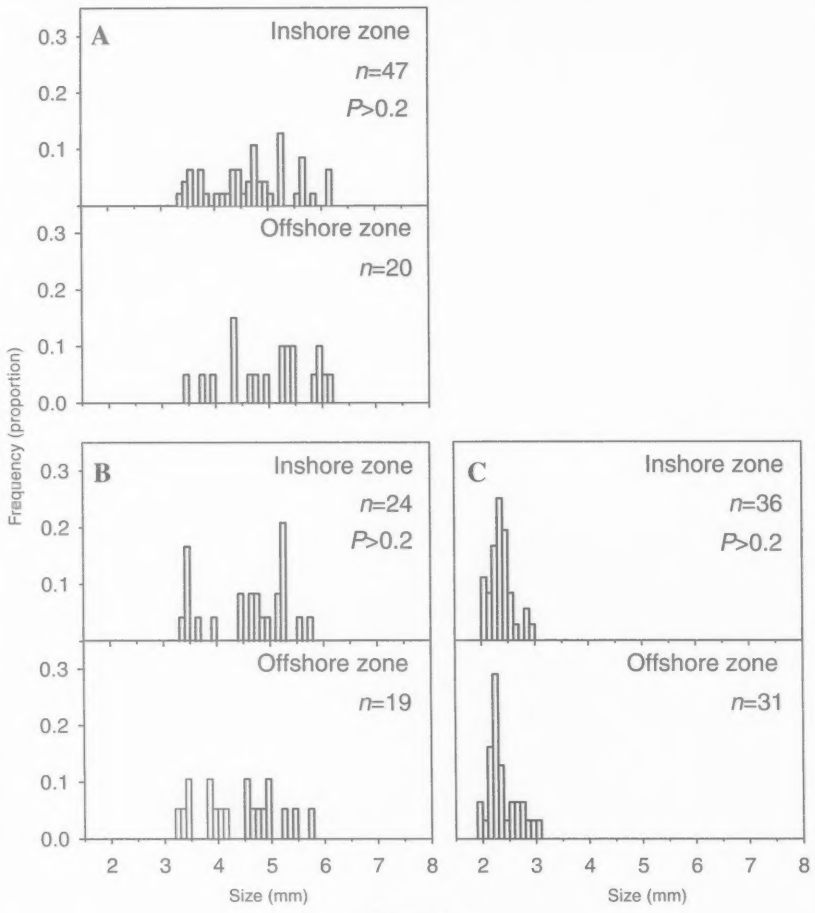

Figure 9

Size-frequency distributions for larvae from inshore and offshore zones of the Coral Sea of *Thunnus alalunga* (albacore) during the (**A**) early November, and (**B**) late November cruises, and for *Auxis-Euthynnus* during the (**C**) early February cruise. Standard length (mm) of larvae was measured to the nearest 0.1 mm. *P*-values refer to the significance of Kolmogorov-Smirnov (K-S) tests conducted between the inshore and offshore zones within a cruise.

near-reef (within 2 km offshore) distributions of *Makaira indica* (black marlin), *M. mazara* (blue marlin), and *Istiophorus platypterus* (Indo-Pacific sailfish) larvae in the Coral Sea ("the anstau hypothesis," Leis et al., 1987). The istiophorid larvae examined by Leis et al. (1987) for horizontal distributions were taken from the same samples used in this study. Like billfish, larvae of *Thunnus* spp. also have relatively shallow distributions; greatest abundances were found in the upper 20 m of the water column around Oahu Island (Boehlert and Mundy, 1994) and higher concentrations were found at 5 m depth than at 10 m depth in French Polynesia (Leis et al., 1991). Larvae of *T. atlanticus* (blackfin tuna) were caught in greatest numbers in the upper 20 m of the water column in the northern Caribbean Sea, and few larvae were caught below 40 m depth (Hare et al., 2001). We cannot confirm this hypothesis, however, because we did not take direct measurements of either currents or the vertical distributions of tuna larvae during the present study period.

Downwelling on the seaward side of the outer reefs in the Coral Sea could account for the simultaneous occurrence of opposing on-offshore distributions of *K. pelamis* and *Thunnus* larvae because of known differences in the vertical distributions of larvae between these two genera. Larvae of *K. pelamis* have deeper distributions

than *Thunnus* spp. larvae (Boehlert and Mundy, 1994; Hare et al., 2001), and migrate into deeper water during the day, at which time larvae of *Thunnus* spp. move into surface layers (Richards and Simmons, 1971; Davis et al., 1990b). It is therefore possible that while *Thunnus* spp. and *T. alalunga* larvae in the present study were advected shoreward by wind-driven surface currents, and accumulated there by a tendency to remain near the surface, larvae of *K. pelamis* were advected offshore by deeper return flow originating from downwelling near the outer reefs. At the least, *K. pelamis* larvae would not accumulate near the reef front because they would not be expected to counter the putative downwelling at those locations.

The larger size and greater abundance of *K. pelamis* larvae offshore indicate that the larvae of this species likely accumulated there, providing support for the hypothesis of offshore physical transport at depth for this species. And although *T. albacares* and *T. alalunga* larvae were not larger inshore, as would be expected if larvae were transported onshore and accumulated near the reef, larvae of these species >3.5 mm SL were common near the reef, which was not the case for *K. pelamis*. It is possible that the size distributions of *T. albacares* and *T. alalunga* larvae in the Coral Sea may have been affected by greater mortality of larger size classes in the inshore zone than in the more offshore waters. It has been hypothesized that predation rates of larval fish are higher in near-reef waters than in the open ocean (Johannes, 1978), and direct observations of late-stage reef fish larvae have shown that larvae near reefs feed less and are preyed upon more often than larvae farther offshore (Leis and Carson-Ewart, 1998).

The patterns of on-offshore distribution of tuna larvae documented here support the hypothesis that at least some tuna species have high larval abundances near reefs in the Tropical Pacific Ocean. We conclude that fine-scale (1–10 km) on-offshore distributions of tuna larvae found in the Coral Sea were most likely the result of relatively near-reef spawning patterns of adults (<10 km offshore) subsequently modified by wind-driven onshore currents and presumed downwelling in front of the outer reefs of the Great Barrier Reef. To account for different horizontal distributions of larvae among taxa, we suggest that putative opposing flow directions between the surface layers and deeper water may have interacted with the taxa-specific vertical distributions of larvae. An investigation of physical and biological factors, vertical distributions of larvae, and the abundance and distribution of spawning adults near reefs is required to further our understanding of the primary causes of on-offshore distributions of tuna larvae.

Regardless of how distributions occurred, near-reef areas may generally be more important than offshore areas for the production of *T. albacares* and *T. alalunga* larvae, and possibly other large pelagic species. It is now evident from four studies that larvae of *T. albacares* and *T. alalunga* are abundant in near-reef (<5 km offshore) waters, and in the two studies where larval tuna abundances near a reef were compared with larval tuna abundance in offshore areas, higher abundances of *Thunnus* spp. larvae were found near the reef (the present study; Boehlert and Mundy, 1994). These studies also indicate that *K. pelamis* may, at least, spawn close to shore, although their larvae are not most abundant there. Larvae of other large pelagics, such as billfishes, may also be generally more abundant near reefs, as indicated by the near-reef abundance of larvae of three species in our study area (Leis et al., 1987). Near-reef areas have not received much attention in studies of distribution and abundance of larvae of large pelagic predators like tunas and billfishes. If the patterns found thus far are a general occurrence in tropical regions, larval abundance surveys that do not include these areas may underestimate true abundances. It must be kept in mind, however, that near-reef areas are much smaller than oceanic areas. Therefore, in spite of higher abundances (per unit of area) of larvae near reefs, the offshore areas may provide the bulk of the recruits to adult populations, because of the vast areas involved. As yet, there are no data on the survival rates of larvae near reefs compared to the survival rates of larvae offshore, or on their relative contributions to spawning populations.

Acknowledgments

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Habitat and bycatch effects on population parameters of inshore lizardfish (*Synodus foetens*) in the north central Gulf of Mexico

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Abstract—We examined the effect of habitat and shrimp trawl bycatch on the density, size, growth, and mortality of inshore lizardfish (*Synodus foetens*), a nonexploited species that is among the most widespread and abundant benthic fishes in the north central Gulf of Mexico. Results of quarterly trawl sampling conducted from spring 2004 through spring 2005 revealed that inshore lizardfish are most abundant on sand habitat, but larger fish are more common on shell rubble habitat. There was no significant difference in fish density between habitats exposed to shrimp trawling on the open shelf versus those habitats within a permitted artificial reef zone that served as a *de facto* no-trawl area; this finding indicates that either inshore lizardfish experienced minimal effects from trawling or, more likely, that fish moved between trawled and nontrawled habitats. Exploitation ratio (bycatch mortality/total morality) estimates derived from catch curve analysis ranged from 0.43 inside the artificial reef zone to 0.55 outside the reef zone, thus indicating that inshore lizardfish are subject to significant fishing mortality in the north central Gulf of Mexico despite the lack of a directed fishery for the species. We infer from this result that effects of shrimp trawl bycatch may be significant at the population level for nonexploited species and that a broader ecosystem-scale examination of bycatch effects is warranted.

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Habitat degradation and bycatch of adult or younger life-stage indviduals of nontargeted species are among the greatest ecosystem effects of fishing (Hall et al., 2000; Pauly et al., 2002, 2003; Thrush and Dayton, 2002). In fact, bycatch of nontargeted species or life stages may pose an even greater threat to marine ecosystem health than direct the harvest of targeted species (Crowder and Murawski, 1998; Baum et al., 2003; Harrington et al., 2005). Therefore, biologists, conservation groups, and fisheries agencies have called for a transition away from single species management to a more holistic ecosystem-based fisheries management (EBFM) approach (Zabel et al., 2003; Francis et al., 2007; Marasco et al., 2007). A single-species approach traditionally has been applied to estimate maximum sustainable yield (MSY) and to subsequently set total allowable catch (TAC) for most fisheries, but that approach does not account for or mitigate against direct and indirect ecosystem effects of fishing.

The shortcomings of single species management are evident when considering management of the penaeid shrimp trawl fishery in the northern Gulf of Mexico (GOM). That fishery has operated near its estimated MSY since the 1950s, averaging approximately 130×10^6 lbs/yr in total landings of brown (*Farfantepenaeus aztecus*), white (*Litopenaeus setiferus*), and pink (*Farfantepenaeus duorarum*) shrimps, but greater than 1×10^9 lbs/yr of bycatch have also been associated with those landings (Diamond, 2004; Ortiz et al., 2000; Strelcheck and Hood, 2007). Although that level of bycatch is clearly substantial, its effects on fishes at the population level have been studied for only a few commercially important fishes (Diamond et al., 2000; Porch, 2007). Perhaps a more comprehensive approach would be to analyze the effects of bycatch on the community (e.g., Wells, 2007), as well as to examine species-specific effects for nonexploited but ecologically important fishes.

Inshore lizardfish (*Synodus foetens*) is an ideal model species for examining the effects of shrimp trawl bycatch on the population demographics and dynamics of an ecologically but not commercially important species. Inshore lizardfish are ubiquitous on the northern GOM shelf and are among the ten most frequently encountered benthic fishes in trawl samples there (Wells, 2007). The ecological importance of inshore lizardfish has been assumed to be significant given their abundance on the shelf and carnivorous habits (Garcia-Abad et al., 1999); however, relatively little is known about their population ecology in the

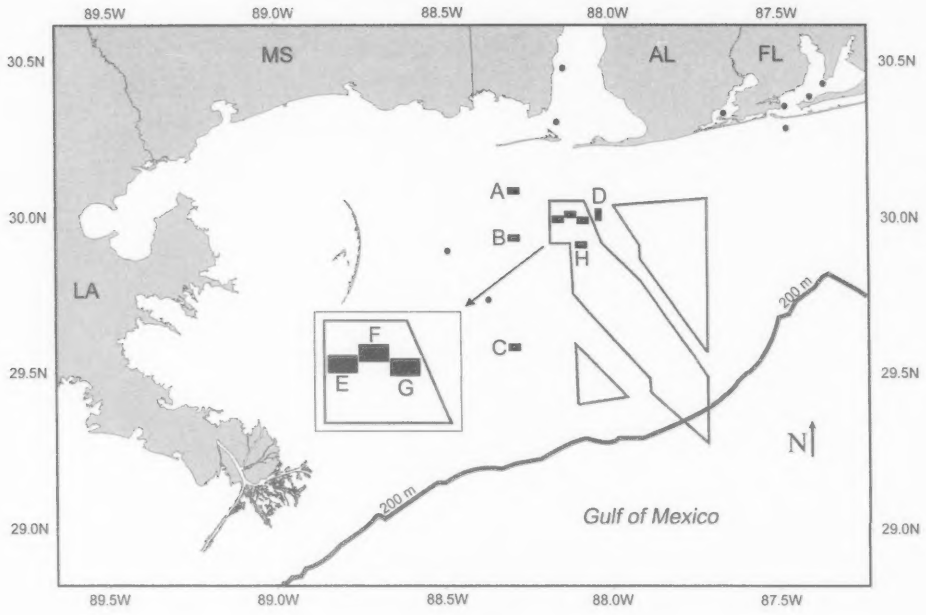

Figure 1

Map of areas sampled for inshore lizardfish (*Synodus foetens*) in the northern Gulf of Mexico from spring 2004 through spring 2005. Large polygons on the shelf indicate artificial reef zones. Habitats inside the artificial reef zones served as *de facto* no trawl areas. Habitats A and F are predominantly sand; habitats B and E are low relief (1–2 m) shell rubble; habitats C and G are high relief (2–3 m) shell habitat; and habitats D and H are reef habitats. Filled circles indicate sites where inshore lizardfish were sampled opportunistically for otolith marginal condition and age analyses. The 200-m isobath is shown to indicate the shelf edge.

northern GOM. As part of a broader study examining the effects of habitat type and shrimp trawl bycatch on demersal and benthic fishes in the north central GOM, the objectives of the work presented here were to examine inshore lizardfish life history and ecology and to estimate potential impacts of shrimp trawl bycatch on them. Specifically, we estimated the effect of habitat type and shrimp trawl bycatch on inshore lizardfish density, size, age, and mortality in various habitats on the north central GOM continental shelf.

Materials and methods

Inshore lizardfish were sampled within four habitat types in the north central GOM: sand, low-relief shell rubble (low shell), high-relief shell rubble (high shell), and reef. Habitats were mapped with sidescan sonar and groundtruthed with boxcores and benthic grab samplers during previous studies (Schroeder et al., 1988; Dufrene, 2005; Strelcheck et al., 2005). Dufrene (2005) characterized shell rubble habitats as ridges standing 1–m (low-relief) or 2–3 m (high-relief) above the surrounding seabed and extending up to 200 m across. Boxcore sediment samples revealed that ridges are composed of >50% calcium carbonate ($CaCO_3$), and shell fragments from both marine and estuarine taxa, the latter of which come from lower sea levels that date from the Holocene Epoch. Reef sites were characterized by Schroeder et al. (1988) as reef-like outcrops of rock rubble and shell hash supporting a diverse epifaunal assemblage. Each of the four study habitat types was found on both the open shelf and within an artificial reef zone (AR zone) off Alabama (Fig. 1). Shrimp trawling is prevalent on the open shelf, but the AR zone functions as a *de-facto* no trawl area (NRC, 2002, Fig. B.9 in that report). Therefore, by sampling inside and outside the AR zone the effect of shrimp trawl bycatch on inshore lizardfish population parameters could be tested.

Inshore lizardfish were sampled quarterly from May 2004 to April 2005 in the habitats described above using a bottom trawl rigged and fished according to the Southeastern Monitoring and Assessment Program's (SEAMAP) trawling protocol, except that trawl samples were not taken during winter and spring 2005 in the high shell habitat outside the AR zone because of weather constraints. The sampling gear was a 12.8-m, four-seam semiballoon otter trawl rigged with 2.4-m ×1-m doors, a 54.9-m bridle, a tickler chain set 1.1 m shorter than the footrope, and a codend with 4-cm

mesh. The sole deviation from SEAMAP protocol was the inclusion of a 0.7-cm mesh bag within the codend to retain small fish. Three replicate tows were made in each habitat type inside and outside the AR zone during quarterly sampling. Before trawl sampling, a hydrographic cast was made at each station with a Seabird 19plus CTD (Sea-Bird Electronics Inc., Bellevue, WA) to measure temperature, salinity, and dissolved oxygen. Then, trawling was conducted at 4.6 km/h for approximately 10 minutes. Up to ten fish were randomly sampled from each trawl sample and immediately frozen in ziploc bags and brought to the laboratory for processing. Individuals were thawed, weighed to the 0.1 g, measured to the nearest mm total length (TL), and their sex was determined by macroscopic examination of gonads. Both sagittal otoliths were then extracted for age analysis as detailed below.

Additional fish were opportunistically sampled with trawls approximately monthly from the north central GOM (Fig. 1). In the laboratory, sampled individuals were measured, weighed, and their sex determined as above. Their sagittae then were extracted and stored. The purpose of opportunistic collections was to provide monthly samples to identify the timing of otolith opaque zone formation by means of marginal condition analysis, but those samples were also included in age and growth analyses.

Inshore lizardfish density was estimated as the number of individuals caught divided by the area swept by the trawl. Trawl width under tow was estimated as 0.8×12.8 m, which was based on National Marine Fisheries Service (NMFS) unpublished data of towing conditions for their standard trawl survey gear. Distance towed was estimated with GPS coordinates recorded while the trawl was on the seafloor. Analysis of variance (ANOVA; 5×4×2 factorial design) allowed us to test sampling quarter, habitat, and exposure to trawling effects, as well as their interactions, on inshore lizardfish density and size. All statistical analyses were computed with SAS (SAS Institute Inc., Cary, NC). Density values were transformed by taking the natural log of (*density* +1) and size was log-transformed to meet parametric assumptions of normality and heteroscadascity. Analysis of variance test statistics (*F*-tests) were computed for both density and fish size with type-III sums of squares despite the two missing cells (i.e., no high shell data outside the AR zone for either winter or spring 2005) in the factorial design noted above. However, additional ANOVAs were also computed after removing all high shell habitat data to confirm results from the unbalanced models computed with the full data set.

One sagitta from each fish was used to determine age. Otoliths were set in epoxy and a 0.5-mm transverse section including the core was cut with a Buehler (Buehler Ltd., Lake Bluff, IL) Isomet saw fitted with a diamond blade. Each section was mounted on a glass slide with thermoplastic cement, sanded with 3200 grit wet-or-dry sand paper, and polished with an alumina powder slurry on a felt polishing cloth. Marginal condition analysis was performed to verify that opaque zones formed annually in adults (Beckman et al., 1990). Opaque zones were then counted to determine age. Otolith thin sections were read with a compound microscope with both reflected and transmitted light. Two different readers counted opaque zones in each sample; readers counted opaque zones independently without knowledge of fish size or the other reader's age estimate to ensure that no bias occurred during process. If counts for a given otolith differed, the otolith was read a second time by both readers. Average percent error (APE) was computed to assess precision between readers (Campana, 2001). Length-at-age was plotted and then least-squares fits of the nonlinear von Bertalanffy growth curve were computed to estimate inshore lizardfish growth for males, females, and both sexes jointly:

$$L_t = L_\infty (1-e^{-K(t-t_0)}), \quad (1)$$

where L_t = estimated length at age t;
L_∞ = asymptotic length;
K = growth coefficient;
t = age in years; and
t_0 = hypothetical age at zero length.

A likelihood ratio test was computed to test whether sex-specific growth functions were significantly different from one another (Kimura, 1980).

Three independent estimates of natural morality (M) were computed based on maximum observed longevity according to the methods of Royce (1972), Hoenig (1983), and Pauly (1980):

$$M = 4.6/(t_{max}) \text{ (Royce, 1972)}, \quad (2)$$

$$\ln(M) = 1.44 - 0.982 \ln(t_{max}) \text{ (Hoenig, 1983)}, \quad (3)$$

$$\ln(M) = -0.0152 - 0.279\ln(L_\infty) + 0.6543\ln(K) + 0.463\ln(T) \text{ (Pauly, 1980)}, \quad (4)$$

where M = natural mortality/yr;
t_{max} = the oldest aged fish in sample; and
T = mean temperature °C.

Total instantaneous annual mortality (Z) was estimated with catch curve analysis (Ricker, 1975). Bycatch mortality (F_b) was computed by subtraction:

$$F_b = Z - M \quad (5)$$

where F_b = instantaneous annual fishing (bycatch) mortality;
Z = instantaneous annual total mortality; and
M = instantaneous annual natural mortality.

The difference in total mortality between habitats exposed to trawling versus nontrawled habitats was tested with analysis of covariance (ANCOVA) test for equal slopes. Exploitation ratio (E) was estimated as the ratio of F_b/Z (Ricker, 1975).

Table 1

Mean environmental parameters measured in habitats sampled for inshore lizardfish, *Synodus foetens*, in the northern Gulf of Mexico during 2004 and 2005 sampling. Habitat C (high relief shell rubble outside the artificial reef zone) was not sampled in winter or spring 2005. Descriptions and locations of habitat types are provided in Figure 1.

Quarter	Sampling dates	Habitat	Depth (m)	Temperature (°C)	Salinity (psu)	Dissolved oxygen (mg/L)
Spring 2004	16 May 04	A	17.3	22.2	34.2	7.0
	16 May 04	B	30.3	19.9	35.2	6.5
	19 May 04	C	39.6	19.5	35.7	6.3
	18 May 04	D	19.0	24.4	34.6	6.9
	17 May 04	E	29.0	20.6	35.2	7.3
	18 May 04	F	27.3	20.7	35.1	7.1
	18 May 04	G	24.0	21.1	35.0	7.5
	17 May 04	H	28.0	20.6	35.2	7.3
Summer 2004	3 Aug 04	A	18.0	23.5	36.0	3.3
	3 Aug 04	B	30.6	22.9	36.4	4.2
	4 Aug 04	C	37.2	20.7	36.4	5.0
	5 Aug 04	D	19.8	24.0	36.0	4.6
	4 Aug 04	E	28.8	22.6	36.1	3.4
	4 Aug 04	F	28.0	22.9	36.1	3.6
	5-Aug 04	G	23.0	22.3	36.2	1.8
	4 Aug 04	H	30.7	22.5	36.1	2.9
Fall 2004	29 Oct 04	A	17.7	26.0	35.5	3.9
	27 Oct 04	B	30.7	23.1	36.3	4.9
	26 Oct 04	C	37.4	28.1	36.2	5.8
	28 Oct 04	D	18.3	26.0	35.3	5.4
	27 Oct 04	E	28.8	27.0	36.3	5.7
	28 Oct 04	F	26.5	26.7	36.3	5.2
	28 Oct 04	G	27.5	25.8	35.8	4.7
	27 Oct 04	H	29.0	26.5	36.3	5.2
Winter 2005	27 Jan 05	A	18.0	20.1	35.6	5.9
	25 Jan 05	B	30.8	20.1	35.6	5.9
	26 Jan 05	D	19.0	18.1	34.5	6.7
	25 Jan 05	E	29.0	20.6	35.7	5.9
	26 Jan 05	F	26.5	20.8	35.7	5.9
	26 Jan 05	G	23.5	19.3	35.2	6.4
	25 Jan 05	H	31.5	19.8	35.5	6.3
Spring 2005	27 Apr 05	A	18.0	20.2	36.3	3.7
	27 Apr 05	B	31.0	20.2	36.3	3.7
	28 Apr 05	D	18.6	20.3	35.5	3.9
	28 Apr 05	E	29.5	20.5	36.0	4.0
	27 Apr 05	F	27.0	20.2	36.3	3.7
	28 Apr 05	G	24.0	20.4	35.9	3.6
	28 Apr 05	H	31.0	20.6	36.0	4.1

Results

All habitat types were sampled each quarter, with the exception noted above that high shell habitat outside the artificial reef zone was not sampled during winter and spring 2005. Hydrographic parameters generally were consistent among sampled habitats within sampling quarters. Water depth of trawl samples ranged from a mean of 18 m for sand habitat outside the AR zone to 38 m for high shell habitat outside the AR zone (Table 1). Temperature ranged from 19° to 26°C among quarters (mean=22.1°C), and salinity ranged between 35 and 36 psu. Dissolved oxygen fluctuated by season and was lowest during summer months.

A total of 1239 inshore lizardfish were caught in trawl samples in study habitats; 749 of those fish were randomly sampled and brought to the laboratory for further analysis. An additional 221 fish were sampled

opportunistically in various locations to enhance sample sizes and the temporal coverage for otolith marginal condition analysis and aging (Fig 1). Within study habitats, inshore lizardfish appeared in 91% of all trawl samples. Fish density was significantly different among sampling quarters and habitats (ANOVA, $P<0.001$ for both), but not between trawled and untrawled habitats (ANOVA, $P=0.2754$; Fig. 2). However, interpretation of the main effects on density is complicated because of significant first-order interactions between sampling quarter and trawl effects (ANOVA, $P<0.001$) and habitat and trawling effects (ANOVA, $P<0.001$); there was no difference in significance for main effects or interactions between tests computed with or without the high shell habitat data. Inshore lizardfish were more abundant in sand habitat (mean density ±standard error [SE]=22.5 [±4.0] fish/ha) than in the other three habitats (mean density <13 fish/ha), but the level of difference was driven by two sand habitat samples taken outside the AR zone in summer 2004 that yielded the highest estimated densities (105 and 65 fish/ha, respectively). Among study habitats not exposed to trawling, inshore lizardfish densities were similarly high **for** sand and high shell habitats (Fig. 2A).

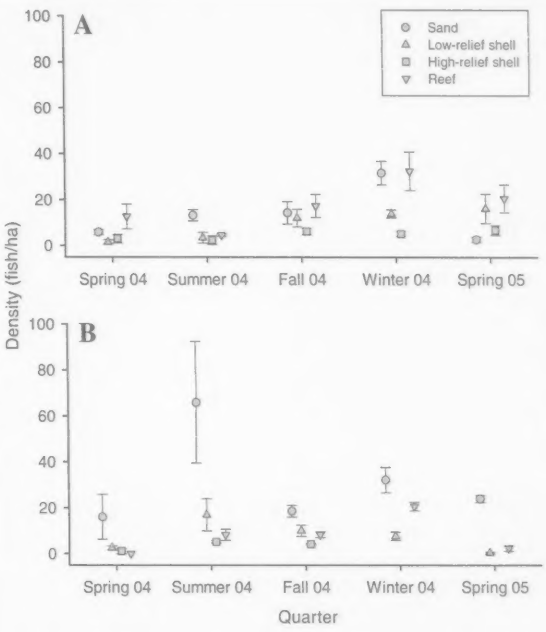

Figure 2

Mean (±standard error) density (fish/ha) of inshore lizardfish (*Synodus foetens*) sampled from spring 2004 through spring 2005 in study habitats in the northern Gulf of Mexico for (**A**) areas inside the artificial reef zone that were not subjected to trawling and (**B**) areas outside the artificial reef zone that were subjected to trawling.

Inshore lizardfish caught during quarterly sampling ranged in TL from 49–404 mm (Fig. 3A), whereas opportunistically sampled fish ranged in TL from 76 to 472 mm (Fig. 3B). Fish length was significantly different among sampling quarters (ANOVA, $P<0.001$), habitats (ANOVA, $P<0.001$), and levels of the trawling effect (ANOVA, $P=0.006$; Fig. 3). However, interactions between sampling quarter and habitat (ANOVA, $P<0.001$) and habitat and the trawl effect (ANOVA, $P<0.001$) complicated interpretation of the main effects; there was no difference in significance for the main effects, or their interactions on fish size between tests computed with and without the high shell habitat data. Overall, mean (±SE) fish size was smaller in habitats exposed to trawling (230.7 [±3.2] mm TL) than in habitats sampled within the AR zone (242.9 [±2.2] mm TL). However, high shell habitat outside the AR zone sampled in spring 2004 had the largest mean (±SE) size (320.0 [±44.4] mm TL) among all factor level combinations, although only three fish were captured in that habitat in spring 2004. Fish size was smallest (mean TL ±SE=204.7 [±4.6] mm) during summer 2004 among all habitats. That trend was most pronounced in sand habitat both inside and outside the AR zone where high densities (Fig. 2) of mostly small fish (Fig. 4) were encountered.

Of the 970 otoliths prepared for age estimation (749 from quarterly sampling and 221 opportunistically sampled), age could be determined for 967. Marginal condition analysis demonstrated that otolith opaque zones generally began forming in November and continued to do so until February (Fig. 5). All samples were at least one year of age. The oldest fish sampled was a 424-mm-TL 9-year-old female that was sampled in June 2006 onboard during the SEAMAP trawl survey. Of the fish sampled within study areas, the oldest was 8 years and nearly half of all fish were 3-year-olds. Reader agreement was judged to be good with an APE of 5.94%.

Von Bertalanffy growth functions were not significantly different between sexes (likelihood ratio χ^2-test, $P=0.998$); thus size-at-age data were modeled jointly between sexes. The resultant growth equation was $L_t=290.8(1-e^{-0.486\,(t-0.204))}$ (nonlinear regression, $P<0.001$; r^2 (coefficient of determination? If so, lowercase)=0.22) (Fig. 6). Estimates of M based on an observed t_{max} of 9 yr were 0.51/yr, 0.49/yr, and 0.53/yr from the methods of Royce (1972), Hoenig (1983), and Pauly (1980), respectively. Before conducting catch curve analysis, the age distribution of the aged fish (n=749) sampled in our study habitats was expanded to the samples collected that were not aged (n=490). This was accomplished by computing habitat- and sampling quarter-specific age distributions and by assigning age by means of a random number table for fish collected that were not aged. Then, catch curve analysis was computed for ages 3 yr and older because those were the fully recruited ages (Fig. 7). Total mortality of inshore lizardfish sampled within the AR zone was Z = 0.93/y

(linear regression, $P<0.001$, $r^2=0.993$); estimated total mortality outside the AR zone was Z = 1.10/yr (linear regression, $P<0.001$, $r^2=0.971$). Therefore, fish outside the AR zone were exposed to higher Z than those inside, but the slopes were not significantly different between the two catch curves (ANCOVA test for equal slopes, $P=0.232$). By subtraction, F_b estimates ranged from 0.40 to 0.44/y inside the AR zone and 0.57 to 0.61/yr outside the AR zone. Resultant E estimates ranged from 0.43 to 0.47 for fish sampled inside the AR zone and 0.52 to 0.55 for fish outside the AR zone.

Discussion

Inshore lizardfish were nearly ubiquitous throughout study areas. Fish abundance was greater in summer, especially for smaller individuals, possibly indicating that recruitment to study habitats from estuarine and inshore habitats occurred in summer. Small inshore lizardfish begin recruiting from estuaries to Campeche Bay in the southern GOM in June and continued to do so until October (Garcia-Abad et al., 1999). A similar pattern was observed in the present study where both size and inshore lizardfish age were lowest in summer. Overall, catch-at-age data indicated that inshore lizardfish did not fully recruit to the study sites until age three, but the mean age of fish sampled in summer was slightly less than 3 yr. Fish size, as well as age, was greatest in high shell habitat, but density was lowest there. Cruz-Escalona et al. (2005) reported that adult inshore lizardfish in the southern GOM preferred sand habitat, and our data indicated a similar trend in the northern GOM. However, smaller fish may avoid more complex habitats, such as shell rubble ridges, because of the presence of predators or increased competition for food.

Fish were larger, on average, inside the AR zone, but fish density was not significantly different inside and outside the AR zone, which may have resulted from the movement of inshore lizardfish among trawled and untrawled habitats. No direct observations of inshore lizardfish movement were made in this study, but individuals are known to move 10s of km as they recruit to adult habitats on the shelf from inshore estuarine nursery habitats (Cruz-Escalona et al., 2005). Other lizardfishes also move significant (10s to 100s of km) distances (Sweatman, 1984; Golani, 1993). Furthermore, experimental closure of areas to bottom trawling in Australia's northern prawn fishery did not yield significant differences in lizardfish density between areas open to trawling and those closed to trawling because fish moved into trawled areas after trawling occurred, thus restoring high densities there (Stobutzki et al., 2003). Therefore, the lack of differences observed in inshore lizardfish density in areas exposed to trawling and those not exposed to trawling during the present study may have resulted from fish moving between trawled and untrawled areas.

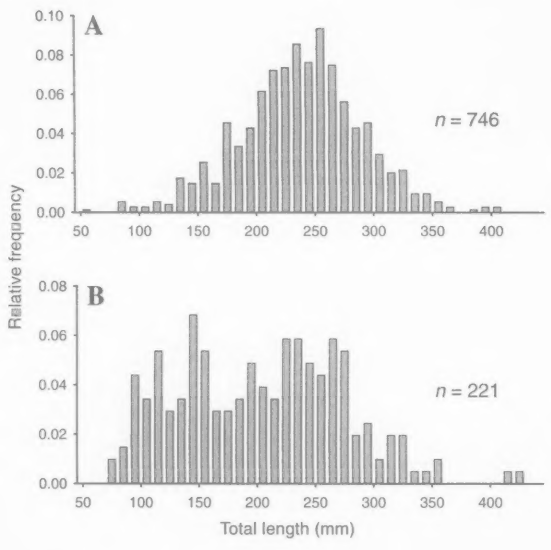

Figure 3

Frequency histograms of total length (mm) distributions of inshore lizardfish (*Synodus foetens*) collected within (**A**) study habitats and (**B**) for fish sampled opportunistically in the north central Gulf of Mexico during spring 2004 through spring 2005. Sample sizes (n) are shown for each historgram.

Marginal conditional analysis confirmed that the timing of opaque zone formation in inshore lizardfish otoliths is similar to a range of demersal (e.g., black drum [*Pogonias cromis*] and red snapper [*Lutjanus campechanus*]) and benthic (e.g., southern flounder [*Paralichthyes lethostigma*]) fishes in the northern GOM (Beckman et al., 1990; Patterson et al., 2001; Fischer and Thompson, 2004) and that annual opaque zones are laid down from November to February. Based on counts of annuli, the maximum longevity observed for inshore lizardfish (9 years) was similar to the maximum longevity reported for other synodontid species. For example, Yoneda et al. (2002) reported that a *Saurida* species from the East China Sea lived to 11 years and Thresher et al. (1986) aged a *Saurida* species from the northwestern Australian shelf to be 7 years.

The low regression coefficient ($r^2=0.22$) of the von Bertalanffy growth function computed with size-at-age data reflects the substantial variability in inshore lizardfish size-at-age. Hood and Johnson (1999) reported vermilion snapper (*Rhomboplites aurorubens*) displayed similar variability in size-at-age in the GOM. They indicated vermilion snapper were difficult to age, but their high reader agreement indicated that the observed variability in size-at-age was not an artifact of inaccurate aging. Similarly, we infer

the variability in size-at-age reported for inshore lizardfish is representative of the variability in the population given the high APE computed between reader age estimates.

Results from catch curve analysis and calculations of F_b appear to indicate that shrimp trawl bycatch mortality was substantial for inshore lizardfish. However, that interpretation depends on the assumption that the sampling gear has a logistic selectivity-at-age function for inshore lizardfish, and that there was no other source of fishing mortality beyond that of shrimp trawl bycatch. If the selectivity-at-age function was domed shaped, for example, then some of the decline in numbers of larger, older fish in our sample would have been due to older fish not being fully selected by the gear; however, our interpretation was that the observed decline, beyond that due to M, was caused by considerable bycatch mortality. Experiments could be designed to estimate the selectivity function of our sampling gear for inshore lizardfish, but no data currently exist to evaluate selectivity. However, given the small size of even the largest inshore lizardfish sampled in relation to the size of the sampling trawl, it seems reasonably safe to assume that a logistic selectivity function existed. Furthermore, it seems unlikely that other sources of fishing mortality, beyond shrimp trawl bycatch, are substantial for inshore lizardfish in the north central GOM. Inshore lizardfish are not targeted by recreational fishermen in the region and we have no knowledge of them being captured in commercial or recreational hook-and-line fisheries as bycatch.

The difference in Z, hence F_b, was not statistically significant between fish sampled inside and those sampled outside the AR zone. Nonetheless, a difference of $Z=0.17$/yr clearly is biologically meaningful. The lack of a larger difference may indicate that either the assumption that no trawling occurs inside the reef zone was false, or that inshore lizardfish moved between habitats inside and outside the AR zone. Results of electronic tracking of shrimp trawler GPS coordinates indicate some shrimping effort may occur inside the AR zone (NRC, 2002, Fig. B.9 in that report). However, shrimping effort was shown to be orders of magnitude greater outside than inside the AR zone. Therefore, the lack of difference in Z, hence F_b, between habitats inside and outside the AR zone most likely was caused by movement of inshore lizardfish into and out of the AR zone.

Exploitation ratios computed for inshore lizardfish appear to indicate that the species is heavily fished in the northern GOM despite the lack of a directed fishery for it. A general rule of thumb is that an E approaching 0.5 indicates that a fished population is fully exploited, whereas a ratio greater than 0.5 indicates heavy fishing pressure

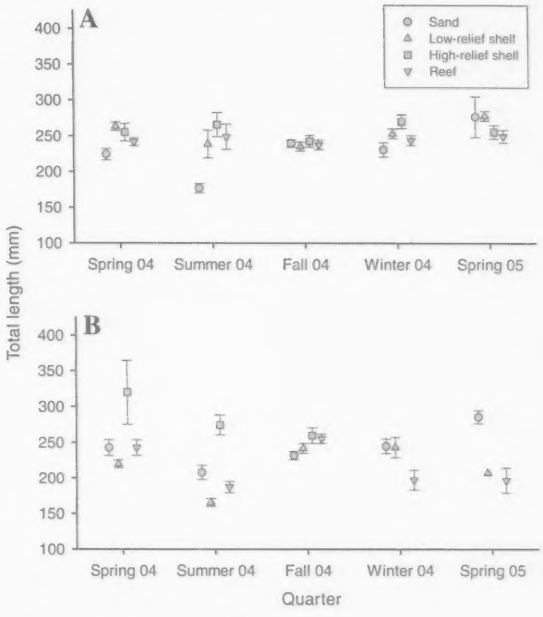

Figure 4

Mean (±standard error) total length (mm) of inshore lizardfish (*Synodus foetens*) sampled from spring 2004 through spring 2005 from study habitats in the northern Gulf of Mexico for (**A**) areas inside the artificial reef zone that were not subjected to trawling and (**B**) areas outside the artificial reef zone that were subjected to trawling.

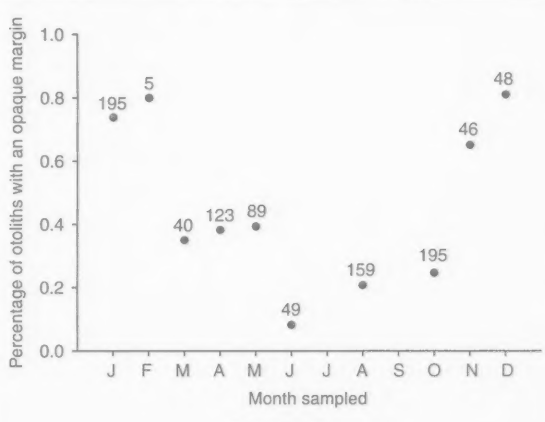

Figure 5

Results from marginal condition analysis of otolith transverse thin sections of inshore lizardfish (*Synodus foetens*). Data plotted indicate the mean number of otolith samples for a given month that had opaque rather than translucent margins. Monthly sample sizes are indicated on the figure.

that could lead to overexploitation (Gulland, 1977). Pauly et al. (2001) demonstrated that ecosystem effects, as measured by declines in the mean trophic level of landings, were apparent at exploitation ratios (E) of 0.4 and were substantial at ratios of 0.6. Silvestre and Garces (2004) estimated E, E_{max} (exploitation rate associated with maximum yield per recruit), and $E_{0.1}$ (a biological reference point analogous to $F_{0.1}$) for 25 fish populations exploited in trawl fisheries in coastal waters of Brunei Darussalam. Fishes were similar in size to inshore lizardfish {mean L_∞ (standard deviation [SD])=262 mm [143]) but their M estimates were much greater (mean M [SD]=1.61/yr [0.48]). Mean (SD) estimates of E_{max} and $E_{0.1}$ among the species were 0.54 (0.04) and 0.36 (0.03), respectively. Our estimates of E_b for inshore lizardfish approach the mean E_{max} level estimated by Silvestre and Garces (2004) and exceed their mean $E_{0.1}$ reference point. Therefore, inshore lizardfish exploitation rates estimated in our study would indicate that the species is fully exploited despite there being no directed fishery for the species. If other non-exploited fishes have similarly high F_b rates as inshore lizardfish, then simulations conducted by Pauly et al. (2001) would indicate that substantial trophic effects of trawl bycatch may exist in the ecosystem.

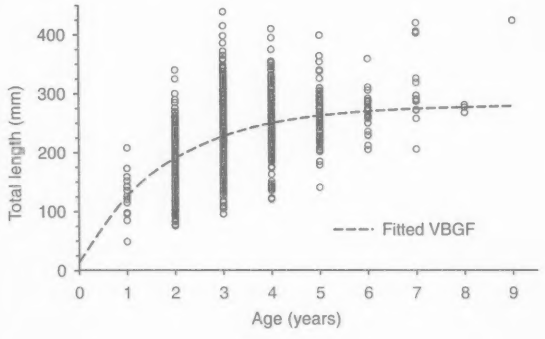

Figure 6

Von Bertalanffy growth function (VBGF) fitted to size-at-age data for inshore lizardfish (*Synodus foetens*), namely all fish (n=967) sampled from spring 2004 through spring 2005 in study habitats in the northern Gulf of Mexico, as well as those sampled opportunistically. Resultant VBGF fitted to all data: $L_t = 290.8(\ 1-e^{-0.486\ (t-0.204)})$, where L_t = length at age t.

Although it is likely that demersal or benthic fishes other than inshore lizardfish have suffered similarly high F_b due to shrimp trawling in the north central GOM, the only other species that have been examined quantitatively (Atlantic croaker [*Micropogonias undulatus*] and red snapper) are also heavily exploited by directed fisheries. Bycatch rates for shrimp trawls have had significant effects on those two species at the population level (Diamond et al., 2000; Porch, 2007; Wells et al., in press), but distinguishing bycatch effects from those caused by the directed fisheries can be problematic. Furthermore, shrimp trawl fisheries have had significant effects on the ecosystem beyond that of bycatch of nontargeted species at various life stages (Thrush and Dayton, 2002; Gray et al., 2006).

In the northern GOM the investigation of bycatch has been centered around the overfished status of red snapper because of the economic importance of that species (Strelcheck and Hood, 2007). Results from this study demonstrate that nonexploited but ecologically important species, such as inshore lizardfish, may be significantly impacted by shrimp trawl bycatch. Results presented here demonstrate that effects of shrimp trawl bycatch may be significant at the population level for nonexploited species and indicate that a broader, ecosystem-scale examination of bycatch effects is needed (Diamond, 2004; Wells et al., in press). If species other than inshore lizardfish show similar direct effects on their populations, or if habitat degradation is causing indirect effects on populations and communities, a new approach to management of the shrimp fishery may be warranted.

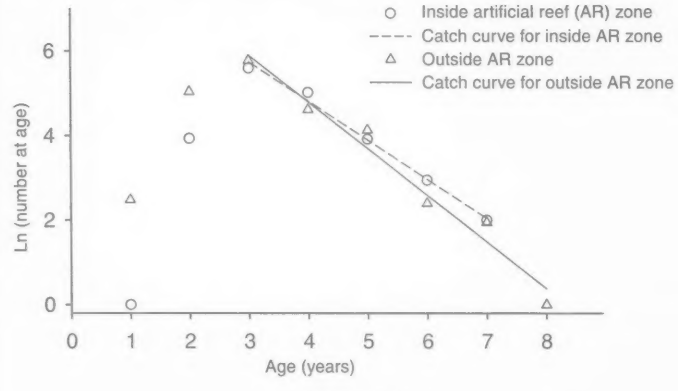

Figure 7

Catch curves computed for inshore lizardfish (*Synodus foetens*) sampled inside (open circles, ○) and outside (open triangles, △) outside the artificial reef zone in the north central Gulf of Mexico from spring 2004 through spring 2005. Plotted lines are linear regressions of ln(*number*) versus *age* for the fully recruited ages: inside the artificial reef zone ln(*catch*) = (−0.94/yr)*age* + 8.18; outside the artificial reef zone ln(catch) = (−1.02/yr)*age* + 8.31.

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Effects of rapid decompression and exposure to bright light on visual function in black rockfish (*Sebastes melanops*) and Pacific halibut (*Hippoglossus stenolepis*)

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Abstract—Demersal fishes hauled up from depth experience rapid decompression. In physoclists, this can cause overexpansion of the swim bladder and resultant injuries to multiple organs (barotrauma), including severe exophthalmia ("pop-eye"). Before release, fishes can also be subjected to asphyxia and exposure to direct sunlight. Little is known, however, about possible sensory deficits resulting from the events accompanying capture. To address this issue, electroretinography was used to measure the changes in retinal light sensitivity, flicker fusion frequency, and spectral sensitivity in black rockfish (*Sebastes melanops*) subjected to rapid decompression (from 4 atmospheres absolute [ATA] to 1 ATA) and Pacific halibut (*Hippoglossus stenolepis*) exposed to 15 minutes of simulated sunlight. Rapid decompression had no measurable influence on retinal function in black rockfish. In contrast, exposure to bright light significantly reduced retinal light sensitivity of Pacific halibut, predominately by affecting the photopigment which absorbs the green wavelengths of light (≈520–580 nm) most strongly. This detriment is likely to have severe consequences for postrelease foraging success in green-wavelength-dominated coastal waters. The visual system of Pacific halibut has characteristics typical of species adapted to low light environments, and these characteristics may underlie their vulnerability to injury from exposure to bright light.

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The effects of events during capture and release on postrelease mortality have been characterized in a range of demersal fish species (e.g., Davis, 2002; Parker et al., 2003; Davis and Parker, 2004; Davis and Schreck, 2005; St John and Syers, 2005; Davis and Ottmar, 2006; Davis, 2007; Hannah and Matteson, 2007; Rummer, 2007), but their impacts on sensory systems have not been addressed. Demersal fishes are commonly subjected to rapid decompression during capture in both commercial and recreational fisheries. The resultant overexpansion of the swim bladder in physoclists causes injuries to multiple organs (barotrauma) including exopthalmia ("pop-eye") (Parrish and Moffitt, 1993; Rummer and Bennett, 2005). Fishes are also commonly held out of the water while being unhooked, released from nets or traps, or sorted from a trawl (Davis and Olla, 2001). During this period they can experience severely impaired oxygen exchange (asphyxia), as well as temperature and light levels above those in their depth range. One or more of the following could, therefore, significantly impair visual function: 1) mechanical damage to the optic nerve or retina resulting from rapid decompression-induced exophthalmia (Rogers et al., 2008); 2) physiological damage to the retinal cells resulting from multiple embolisms, low blood oxygen levels in the choroid rete, avascularity of the retina, and the need of a continuous supply of oxygen in the retinal cells (Fonner et al., 1973; Berenbrink et al., 2005); 3) permanent bleaching of retinal cell photopigments or retinal cell apoptosis (cell death) due to exposure to bright light (Loew, 1976; Wu et al., 2006); and 4) clouding of the cornea or lens (cataract formation) (Midtlyng et al., 1999). Reduced visual function could subsequently result in increased mortality due to predation (Mesa et al.,1994) or the diminished ability to capture prey (Stoner, 2003).

Black rockfish (*Sebastes melanops*) and Pacific halibut (*Hippoglossus stenolepis*) are good representative species for testing the consequences

of events during capture on sensory function. They are commercially and recreationally important demersal species caught by hook-and-line and trawl gear along the west coast of North America. Because of stringent fisheries regulations, both are frequently discarded (Pacific Fishery Management Council, 2004; Hannah et al., 2008). Black rockfish are a deep dwelling physoclist species (Parker et al., 2008) and experience significant barotrauma when brought to the surface, including severe exophthalmia (Hannah and Matteson, 2007; Hannah et al., 2008). Pacific halibut lack a swim bladder and do not suffer apparent injuries associated with rapid decompression. Pacific halibut are, however, often kept on deck of for 30–60 min during sorting operations on commercial trawl vessels before being discarded (Davis and Olla, 2001). This time onboard results in prolonged exposure to air and direct sunlight. Exposure to bright light causes photoreceptor damage in both vertebrates and invertebrates (Meyer-Rochow, 1994; Wu et al., 2006); and fishes that normally live in dim light environments are predicted to be especially prone to light-induced damage (Loew, 1976).

Our objective was to examine changes in visual function in black rockfish subjected to rapid decompression and in Pacific halibut exposed to simulated sunlight with electroretinography. The electroretinogram (ERG) represents the summed potentials of various cell types within the retina, not just those of the photoreceptor cells. Nevertheless, the ERG is considered a useful technique for addressing questions about visual function, including the identification of gross visual deficits (Brown, 1968).

Materials and methods

Black rockfish (37.6–46.3 cm total length [TL]) were captured by hook-and-line in waters off shore of Newport, OR (44°33′N, 124°6′W). Efforts were made to collect fish from as shallow a depth as possible (<25 m), and only individuals showing minimal signs of barotrauma (as defined in Hannah and Matteson, 2007) were retained. Fish were transported to the Hatfield Marine Science Center (Newport, OR). During transport, excess swim bladder gases were reduced by inserting a sterile hypodermic needle through the body wall into the swim bladder in any fish that were positively buoyant or that showed an inability to maintain vertical orientation. In order to be able to identify individuals, all fish were tagged with 12 mm×2.1 mm Destron-Fearing ISO FDX-B 134.2-kHz passive integrated transponder (PIT) tags (Destron-Fearing, St. Paul, MN). Tags were placed in the hypaxial musculature (ventral to the cleithrum at a depth of 0.5–1.0 cm) by using a 12-guage stainless steel veterinary needle and a modified syringe as described by Parker and Rankin (2003).

Fish were held for six months in a fiberglass tank (3.1 m diameter, 1.0 m depth) before use in experiments. The holding tank was supplied with seawater (flow rate: 20 L/min, temperature: 10–12°C, salinity: 30–32‰) and maintained under dim fluorescent light (0.01 µmol photons/m^2/s) with a 12-hour photoperiod. Fish were fed *ad libitum* three times per week with a mixture of chopped frozen clams (*Spisula solidissima*), squid (*Loligo opalescens*), capelin (*Mallotus villosus*), and silversides (*Menidia menidia*).

Age-0 Pacific halibut (31.8–40.2 cm TL) were captured with a beam trawl from Chiniak Bay, Kodiak Island, AK (57°40′N, 152°30′W) and shipped to the Hatfield Marine Science Center. They were subsequently reared for two years before use in experiments in fiberglass tanks (3.1 m diameter, 1.0 m depth) supplied with seawater (flow rate: 20 L/min, temperature: 8–10°C, salinity: 30–32‰). The tanks were maintained under dim fluorescent light (0.01 µmol photons/m^2/s) with a 12-hour photoperiod. Fish were fed *ad libitum* three times per week during the first year and twice per week during the second year with a gel food consisting of squid, Pacific herring (*Clupea pallasii*), pelleted food, krill (*Euphausia superba*), amino acid supplements, vitamins, and gelatin.

Rapid decompression experiments with black rockfish

The rapid pressure change (of 3 atmospheres absolute [ATA]) to which black rockfish were subjected was chosen to mimic the changes in depth that fish encounter during capture by both commercial and recreational fisheries, and which have been shown to cause significant barotrauma (Parker et al., 2006, 2008). Pressure changes were achieved using a large flow-through hyperbaric aquarium system capable of maintaining two groups of fish separately at up to 4.25 ATA. This system (described by Parker et al. [2006]) produces the same signs of barotrauma in captive fish as those observed in wild fish subjected to equivalent pressure changes when being brought up from depth. Two experimental trials were performed. For each trial, three control and four treatment fish were placed in the hyperbaric chambers and acclimated to 4.0 ATA over a period of seven days (Fig. 1). Treatment fish were subjected to a simulated capture scenario consisting of a 3.0-ATA pressure drop over 90 seconds. Individuals were removed from the hyperbaric chamber with a dip net, identified, assigned a score to quantify the external signs of barotrauma, and then immediately placed back in the chamber for repressurization. These procedures resulted in a simulated surface interval (i.e., at 1 ATA pressure) of 10 min, but only a brief period (<1 min) out of the water. Treatment fish were then repressurized to 4.0 ATA over 30 seconds simulating the return to depth after release. Fish were held at 4.0 ATA for an additional 20 hours after the rapid decompression event, then gradually brought to 1.0 ATA according to the decompression schedule (Fig. 1) developed by Parker et al. (2006). To account for any effects of the chamber not associated with rapid pressure change, control fish were subjected to an identical schedule of gradual pressure changes, but not the rapid decompression event. After completion of all pressure changes, fish were removed from the chambers with a

dip net and placed in circular polyethylene tanks (2.0 m diameter, 0.8 m depth) for up to three days before evaluation of visual function. These holding tanks were supplied with seawater (flow rate: 8 L/min, temperature 10–12°C, salinity: 30–32‰) and maintained under dim fluorescent light (0.01 µmol photons/m^2/s) for a 12-hour photoperiod. Fish were not fed during this period.

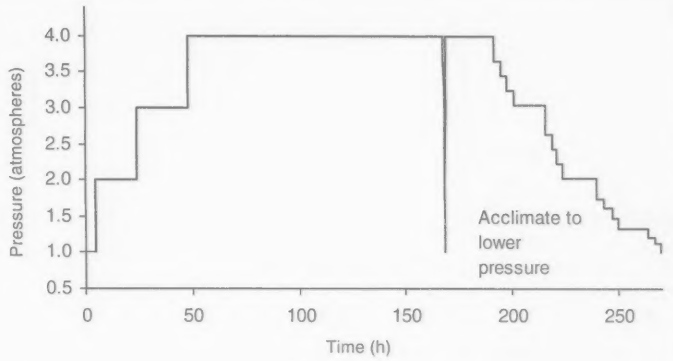

Figure 1

A schematic graph of the time course of pressure changes to which black rockfish (*Sebastes melanops*) were subjected in flow-through hyperbaric aquaria. Control and experimental fish received the same treatments, with the exception that control fish were not subjected to the rapid decompression and recompression shown at approximately 160 h (simulating capture and subsequent return to depth). Time zero indicates the point at which the fish were initially placed in the chamber.

Bright light exposure experiments with Pacific halibut

Individual Pacific halibut were removed with a dip net from their holding tank and held in a seawater bath (12°C). A high intensity xenon lamp (CVI Laser Spectral Products, Albuquerque, NM) was used to produce simulated sunlight. The spectral range of the lamp (≈320–700 nm) approximated the visible (400–700 nm) plus UV range of sunlight at sea level when the sun is directly overhead (Lalli and Parsons, 1997). Light, conducted by fiber optic guide, was aimed at one eye of the fish for 15 minutes. Light intensity at the exit of the fiber optic light guide was 2000 µmol photons/m^2/s (measured over the visible spectral range of 400–700 nm) and matched the intensity of sunlight (2010 µmol photons/m^2/s) measured at Newport, OR, under a clear sky at 12:00 noon PST on 5 October 2007. The 15-minute period of exposure to simulated sunlight was chosen to mimic the minimum time that fish would be left on deck during sorting operations on commercial trawl vessels (Davis and Olla, 2001; Davis and Schreck, 2005). Control fish were treated in the same manner except that the light source was not turned on.

Evaluation of visual function

Fish were maintained under dim light conditions during transport, weighing, and drug injection procedures. They were anesthetized with ketamine hydrochloride (Ketaset, Butler Animal Health, Middletown, PA, dose≈ 30 mg/kg) injected intramuscularly and paralyzed with the neuro-muscular blocking drug gallamine triethiodide (Flaxedil, Sigma Chemical Co., St. Louis, MO, dose≈20 mg/kg) injected directly into the caudal vein. Additional doses of the drugs were administered during the course of an experiment. Fish were euthanized at the conclusion of the experiment with a massive overdose (>300 mg/kg) of sodium pentobarbital (Beuthanasia-D, Schering-Plough Animal Health Corp., Union, NJ) injected intramuscularly.

After drug injections, individual fish were moved into a light-tight enclosure and placed on a perforated rubber sling stretched across an acrylic box. The majority of the body was submerged; only a small portion of the head and the eye receiving the light stimulus remained above the water. The box was supplied with running seawater (12°C) and a small submersible pump continuously circulated water over the gills of the fish. Fish were allowed to acclimate to the dark for at least one hour before any measurements were taken.

Silver-silver chloride electrodes, constructed from teflon-coated silver wire, were used for recording the ERGs. The active electrode was lightly placed on the corneal surface and the reference electrode either in one of the nares or on the skin over the head. (Electrodes were positioned under dim red light [peak wavelength 660 nm] produced by light-emitting diodes [LEDs].) The recording system was grounded to the seawater through a stainless steel plate. ERG signals were amplified by using a 10,000× gain with 1 Hz high pass and 1 kHz low pass filter settings (amplifier model DAM 50, World Precision Instruments, Sarasota, FL). The resultant signal was further filtered with a Humbug® active electronic filter to remove 60 Hz noise (Quest Scientific, North Vancouver, BC, Canada), and digitized at 1 kHz sampling frequency with a multifunction data-acquisition card (model 6024E, National Instruments, Austin, TX). Data recording and stimulus presentations were controlled by a custom-designed software developed by Eric Warrant (University of Lund, Lund, Sweden) using the LabVIEW graphical programming system for measurement and automation (National Instruments, Austin, TX). In order to account for any influence of circadian rhythms on visual responses (Mangel, 2001), experiments were conducted during the hours the fish holding tanks were lit (herein referred to as "day"), and then repeated on the same individual during the hours the fish holding tanks were in darkness (herein referred to as "night"). As a result, the night experi-

ments on Pacific halibut were conducted approximately 10–12 hours after exposure to bright light.

Three separate procedures were conducted to test for treatment effects on visual function: responses to increasing light intensities (V-log I response curves), flicker fusion frequency, and spectral sensitivity. For the first two procedures, light stimuli were produced by a circular (3.8 cm diameter) light source (model SL2420, Advanced Illumination, Rochester, VT) comprising 20 white LEDs. The LEDs were mounted behind a thin diffuser and collimating lens to produce an even (±10% edge to edge) illumination field. Light output was controlled by an intensity controller (model CS410, Advanced Illumination, Rochester, VT), which in turn was controlled the analog output of the data acquisition card. A series of neutral density filters (Kodak Optical Products, Rochester, NY) were used to extend the range of light levels as needed.

For the determination of spectral sensitivity, the output of a xenon fiberoptic light source (model Y1603, CVI Laser Spectral Products, Albuquerque, NM) was controlled with a monochrometer (model CM110, CVI Laser Spectral Products, Albuquerque, NM), two filter wheel assemblies (model AB301, CVI Laser Spectral Products, Albuquerque, NM) containing quartz neutral density filters, and an electronic shutter (model LS6, Uniblitz, Vincent Associates, Rochester, NY). The monochrometer produced single wavelength light (with an 8 nm 50% bandwidth) between 300 and 700 nm. The filter wheel assemblies allowed the attenuation of light from 0 to 5 log units in 0.2 log-unit steps. The monochrometer and filter wheel assemblies were controlled by using serial (RS232) interfaces, and the shutter was controlled by the digital output of the data acquisition card. Light from the xenon light source was conducted through the monochrometer → filter wheel → shutter assembly, and from there directed at the eye through 3-mm light guides. The LED light source and exit point of the light guide were placed approximately 5 cm from the corneal surface. The outputs of the LED and xenon light sources (the latter measured at exit of the light guide after passage through the monochrometer → filter wheel → shutter assembly) were calibrated with a research radiometer (model IL 1700, International Light, Inc., Newburyport, MA).

To construct V-log I response curves, light intensities were increased in 0.2 log-unit steps from levels that produced no measurable responses, to those that produced maximal responses. Stimuli consisted of a train of five 200-ms duration light flashes 200 ms apart. The trains of stimuli at each light intensity were presented every five seconds and repeated five times. The ERG responses to the final flash of each train were recorded and averaged. The data were subsequently normalized by expressing the average response to an intensity step as a fraction of the maximum observed average response. Normalized ERG responses versus log light intensities (candela/meter^2) were plotted to construct V-log I response curves. Mean V-log I curves for each species were constructed by averaging the normalized curves of all individuals within a treatment group recorded during the day or during the night.

Flicker fusion frequencies were determined by using five-second sinusoidal light stimulus trains, followed by five seconds of darkness. The maximum light intensity of the sinusoidal stimulus was that required to produce a response that was 50% of the maximal response. This value was determined by eye during experiments from the individual V-log I response curves. Stimulus trains were repeated five times at each frequency and the responses averaged. Stimulus frequencies were increased from 1 Hz (0 log units) to 63.1 Hz (1.8 log units) in 0.2 log-unit steps. The flicker fusion frequency was determined by comparing the power spectrum of the averaged response at each stimulus frequency (signal) to the power spectrum of a neighboring frequency (noise) and was defined as the frequency at which the power of the signal fell below the power of the noise.

Spectral response curves were determined using monochromatic light flashes produced by a xenon light source and monochrometer → filter wheel → shutter assembly. Approximately isoquantal light stimuli from 300 to 650 nm, regulated by the monochrometer and neutral density filters, were presented to subjects in 10-nm wavelength steps. Five stimuli of 40 ms duration were presented at each wavelength, and five seconds were allowed between each light flash. The responses to the five flashes were averaged and mean amplitudes recorded.

Spectral sensitivity curves were subsequently calculated from the spectral response curves as follows. First, spectral response data were corrected for differences in lamp output at specific wavelengths, as well as for differences in neutral density filter values from their nominal values, through the application of correction factors. The correction factors were based on the calibration curves developed with the research radiometer described above. Second, responses to exactly isoquantal intensities were predicted by adjusting the response amplitude at each wavelength by using spectral V-log I response curves generally following the methods described in Coates et al. (2006). The only exception was that a fourth-order polynomial was fitted to the individual spectral V-log I response data. Spectral V-log I response curves were recorded immediately after measurement of the spectral response curves by using a series of increasingly intense flashes at the wavelength which generated the largest response. Five flashes (200 ms duration, five seconds apart) were delivered at each intensity step and the response amplitudes averaged. Intensities increased in 0.2 log-unit steps and ranged from those producing no response, to those producing a maximal response.

Individual spectral sensitivity curves were normalized by using the maximal response at each wavelength. Mean spectral sensitivity curves were constructed by averaging the spectral sensitivity curves of all individuals within a treatment group recorded during the day or during the night

Table 1

Mean (and 95% confidence bands) intensities (log candela/meter^2) of broad spectrum white light required to produce electroretinogram (ERG) responses 50% of the maximal observed responses in black rockfish (*Sebastes melanops*) and Pacific halibut (*Hippoglossus stenolepis*). The 50% response points were calculated to explore day-night differences, species-specific light sensitivities, and treatment effects. The mean 50% response point in control Pacific halibut was more than an order of magnitude lower than that of black rockfish, indicating the significantly greater light sensitivity of the former. There was no effect of rapid decompression on the 50% response point of black rockfish, whereas 15 minutes of exposure to simulated sunlight increased the 50% response points of Pacific halibut, clearly indicating a diminished light sensitivity in the retina.

	Day	Night
Black rockfish, control	2.1 (2.1–2.2), n=7	2.0 (1.8–2.1), n=7
Black rockfish, rapid decompression	2.3 (2.2–2.3), n=6	1.9 (1.8–2.0), n=6
Pacific halibut, control	0.09 (0.01–0.19), n=6	−0.07 (−0.16 −0.05), n=5
Pacific halibut, light exposed	0.56 (0.47–0.63), n=5	0.78 (0.61–0.91), n=5

Statistical procedures

To explore species differences and treatment effects on retinal light sensitivity, the Weibull four-parameter sigmoid function contained within SigmaPlot for Windows version 10 (Systat Software, San Jose, CA) were fitted to the mean V-log I response data. This function was chosen because ERG responses generally exhibit a sigmoid response to increasing light intensities (Kobayashi, 1962). Light intensities required to produce responses that were 50% of that needed to produce a maximal response (and their 95% confidence bands) were taken from the predicted values produced by the curve-fitting program.

Differences in the mean flicker fusion frequency data collected during the day and night were tested with paired-t tests (SigmaStat 3.1, Systat Software, San Jose, CA) because they represented "before-and-after" trials on the same animal. In those instances where no differences were found, day and night data were combined. Differences in the flicker fusion frequency between control animals and those subjected to rapid decompression (black rockfish) or simulated sunlight (Pacific halibut), were likewise treated with t-tests. In instances where no treatment effects were present, data were combined. The Mann-Whitney rank sum test was used to test for species differences because the combined data sets were not normally distributed. In all instances $P<0.05$ was taken to indicate significant differences.

To explore the specific effects of bright light exposure on retinal function in Pacific halibut, the vitamin A1 rhodopsin absorbance templates developed by Stavenga et al. (1993) were fitted to the normalized spectral sensitivity data assuming the presence of two visual photopigments. Unknown model parameters (photopigment absorption maxima and their weighting proportions) were estimated by using maximum likelihood within the software package *R* (vers. 2.7.0, R Foundation for Statistical Computing, Vienna, Austria).

Results

Effects of rapid decompression

Five of eight black rockfish exposed to simulated capture showed severe exophthalmia immediately after rapid decompression, and one fish in this condition also displayed corneal emphysemas. All eight fish showed other external signs of barotraumas, such as gas bubbles under the branchiostegal membrane, and seven of the eight showed esophageal eversion. None showed any gas bubbles within the vitreous humor. Exophthalmia and other signs of barotrauma disappeared in all fish immediately after recompression. There were no obvious external anatomical abnormalities or evidence of disease in any of the fish at the time they were used in an experiment.

Responses to increasing light intensities

The amplitude of ERG responses of both black rockfish and Pacific halibut increased with increasing light intensities (Fig. 2) and produced the expected sigmoid V-log I response curves (Kobayashi, 1962). Based on the overlap of mean 50% response points and the 95% confidence bands (Table 1), there were no significant day-night differences in the light sensitivity of either species within treatments with the possible exception of black rockfish exposed to rapid decompression. The mean 50% response points in the control Pacific halibut were more than an order of magnitude lower than those of the black rockfish (Table 1), indicating a significantly greater light sensitivity in the former.

There was no effect of rapid decompression on the 50% response point for black rockfish. In contrast, the responsiveness of Pacific halibut retinas was significantly diminished by exposure to bright light. During both day and night recordings, the V-log I response curves for Pacific halibut exposed to simulated sunlight were clearly right-shifted compared to controls (Fig. 2). The mean 50% response points for treated

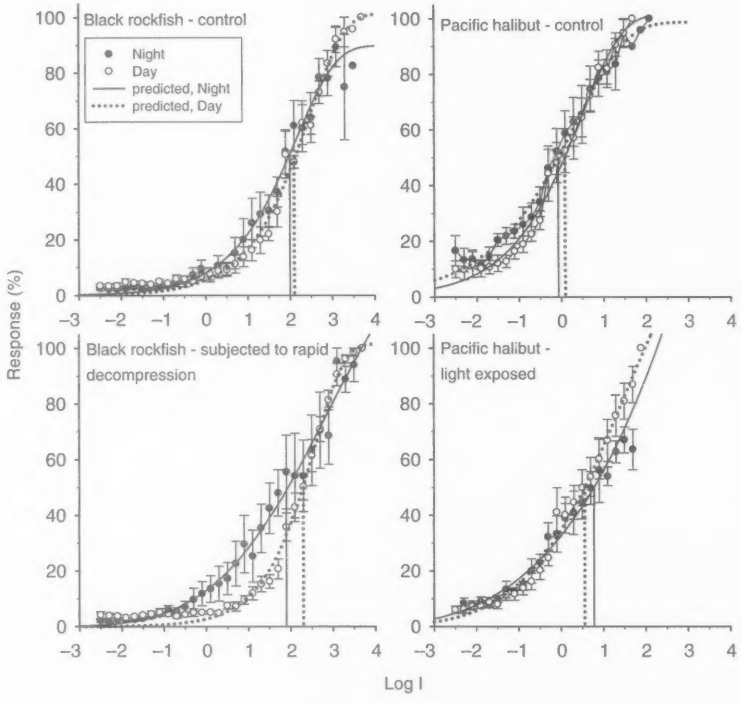

Figure 2

Electroretinogram (ERG) responses to increasing light intensities (I, in log candela/m^2) in black rockfish (*Sebastes melanops*) and Pacific halibut *(Hippoglossus stenolepis*). To construct response curves, ERG responses to 200-ms duration light flashes were recorded as light intensity from a white LED light source was increased in 0.2 log-unit steps from levels that produced no measurable responses, to those that produced maximal responses. Responses from individual trials were normalized by expressing them as a fraction of the maximal response. Data recorded at light levels beyond those that produced a maximal response have been omitted. Results obtained during the day and during the night were analyzed separately. Data points are means ±standard error; open circles show results obtained during the day and filled circles results obtained during the night. The Weibull four-parameter function contained within SigmaPlot for Windows vers. 10 (Systat Software, San Jose, CA) was fitted to the mean V-log I response data. A sigmoidal curve was chosen because the ERG responses generally approached zero at low light intensities, became saturated (i.e., approached a maximum) at high light levels, and varied in between. Dotted lines show the predicted values based on data collected during the day and solid lines show predicted values based on data collected during the night. For illustrative purposes, vertical dotted and solid lines have been added showing the light intensities required to produce responses 50% of the maximal response during the day and night, respectively.

fish were approximately 0.5 to 0.8 log units higher than the mean values for control fish (Table 1). In other words, the light levels required to reach the 50% response points in Pacific halibut exposed to simulated sunlight were three to seven times higher than the light levels required to reach the 50% response points in control fish.

Flicker fusion frequency

There were no day-night differences in the flicker fusion frequencies for either black rockfish or Pacific halibut. There was also no detectable influence of rapid decompression on flicker fusion frequency for black rockfish, nor an apparent effect of exposure to bright light on

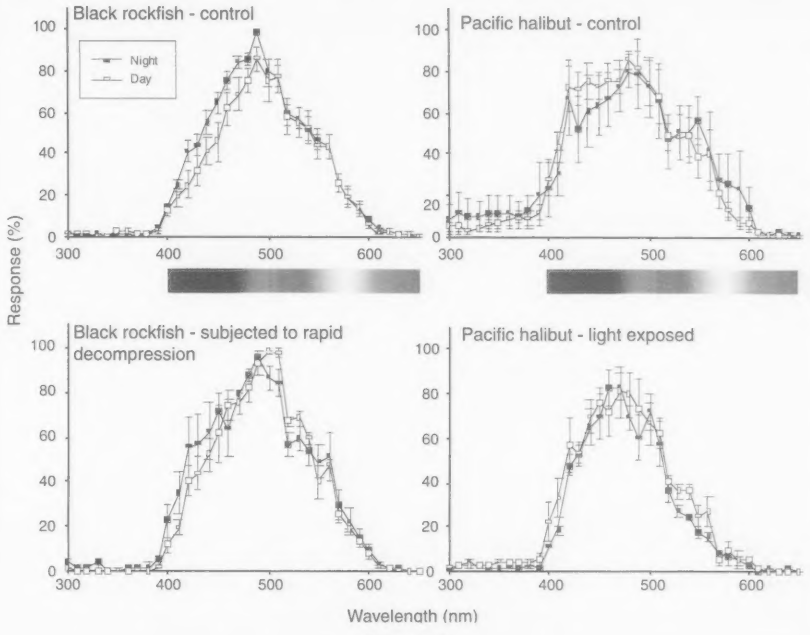

Figure 3

Spectral sensitivity curves for black rockfish (*Sebastes melanops*) and Pacific halibut (*Hippoglossus stenolepis*) derived from electroretinogram (ERG) responses to 40-ms flashes of incremental wavelengths (10-nm steps). Responses from individual trials were normalized by expressing the response to a given wavelength as a fraction of the maximal response recorded during that trial. Data points are means ±standard error. The spectral response curves of black rockfish were unaffected by rapid decompression, whereas the ERG responses of Pacific halibut to green wavelengths (≈520–580 nm) were diminished by 15 minutes of exposure to simulated sunlight. Color bars (truncated at 400 and 650 nm) have been added to illustrate the approximate spectral colors corresponding to the various wavelengths.

the flicker fusion frequency of Pacific halibut. Within-species data were therefore combined to test for cross-species differences. The median flicker fusion frequency for Pacific halibut (30 Hz) was significantly lower than that of black rockfish (49 Hz) ($P<0.001$, Mann-Whitney rank sum Test).

Spectral curves

The spectral sensitivity curves of black rockfish and Pacific halibut were very similar (Fig. 3). Both species showed strong sensitivity to blue-green wavelengths (480–590 nm), and a range of responses from 380 nm (violet) to 610 nm (orange). There was no appreciable sensitivity to the shorter (UV-A, 350–380 nm) nor longer (red, >620 nm) wavelengths in either species.

There was no indication that the spectral sensitivity of black rockfish was affected by rapid decompression (Fig. 3), whereas the spectral sensitivity of Pacific halibut was clearly influenced by exposure to simulated sunlight (Fig. 3). Vitamin A1 rhodopsin absorbance templates developed by Stavenga et al. (1993) were, therefore, used to further assess these changes. In all cases, the templates provided reasonable fits to the observed ERG data (Fig. 4). The parameters showing the largest effects of bright light exposure were clearly the ratios of the long wavelength to short wavelength photopigment weighting factors that are indicative of the relative value of the two photopigments to the composite curve. In control Pacific halibut, this ratio ranged from 0.75 during the day to 0.62 during the night, indicating only a slight predominance of the shorter wavelength photopigment (Fig. 4). In contrast, in Pacific halibut exposed to simulated sunlight these ratios were 0.48 and 0.12 during the day and night, respectively. These results indicate a much reduced functional importance of the longer wavelength photopigment, especially during the night (i.e., approximately 12 hours after exposure to simulated sunlight).

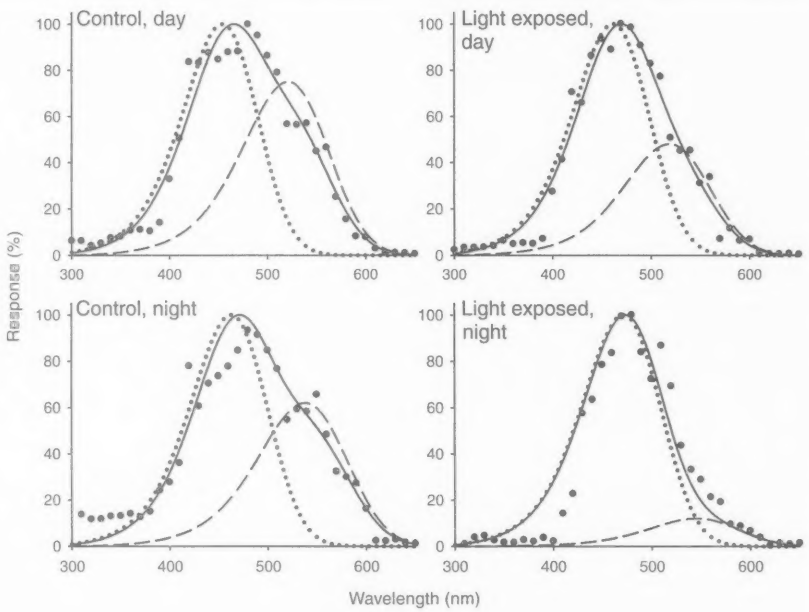

Figure 4

Vitamin A1 rhodopsin absorbance templates (Stavenga et al., 1993) fitted to Pacific halibut *(Hippoglossus stenolepis)* electroretinogram (ERG) data by the maximum likelihood method. The circles show the normalized spectral sensitivity data and the solid lines show the predicated combined photopigment absorption curves. The predicted individual photopigment absorption curves for the short and long wavelength pigments are shown the by the dotted and dashed lines, respectively. The predicted absorption maxima are in reasonable agreement with family Pleuronectidae (Evans et al., 1993; Jokela-Määttä et al,. 2007). Note the dramatically diminished importance of the longer wavelength pigment in halibut exposed to 15 minutes of simulated sunlight, especially at night. The spectral colors corresponding to the various wavelengths are shown in Figure 3.

Discussion

Our observations with black rockfish do not support our original contention that exophthalmia and other internal events associated with rapid decompression compromise retinal function. We found no differences in light sensitivity (V-log I response curves), flicker fusion frequency, or spectral sensitivity between control and experimental fish. Our procedures would not, however, detect damage to the optic nerve or other parts of the central nervous system associated with vision. Experiments involving visually evoked potentials (Bullock et al., 1991), predator- and prey-sighting distance, the optomotor response, or other behavioral procedures (Douglas and Hawryshyn, 1990; Vogel and Beauchamp, 1999; Herbert and Wells, 2002) are needed to confirm the lack of detrimental effects of barotrauma on visual function in black rockfish. Rockfish simply discarded on the surface after being subjected to rapid decompression during capture frequently float. They are than subjected to high rates of avian predation. Returning rockfish to depth (repressurization) reverses the external signs of barotrauma (Parker et al., 2006). Our observations confirm that releasing rockfish at depth is an effective procedure for minimizing postrelease mortality, except for fish with severe hook injuries (St. John and Syers, 2005).

In contrast to the apparent lack of effects of barotrauma on vision in black rockfish, there are multiple lines of evidence that 15 minutes of exposure to simulated sunlight dramatically affects the visual function of Pacific halibut. First, exposure to bright light causes a large reduction in sensitivity (Table 1) indicated by the approximate 3 to 7× increase in amount of light required to achieve an ERG response to broad spectrum white light that is 50% of the maximal response (Table 1). We strongly suspect that low-light vision was also affected. Measuring low-light sensitivity (i.e., minimal detectable ERG response) requires a specific set of procedures different from the ones we employed (Reilly and Thompson, 2007) and we did not have sufficient support for this project to allow us to

conduct the requisite experiments. Pacific halibut are less able to detect baits in near total darkness than at brighter light levels (Stoner, 2003). By extension, we conclude that individuals whose visual function has been compromised by exposure to bright light will be less able to feed and avoid predators than normal animals. Behavioral tests quantifying the effects of bright light exposure on the ability of Pacific halibut to locate and capture prey or detect predators are clearly warranted.

To the best of our knowledge, there are no published descriptions of retinal anatomy in Pacific halibut. The retinas of Atlantic halibut (*Hippoglossus hippoglossus*) contain both rods and cones (Kvenseth et al., 1996), and we strongly suspect that the retinas of Pacific halibut do also. Likewise, we know of no microspectrophotometry studies detailing the absorbance maxima of photopigments in juvenile or adult Pacific halibut retinas. Microspectrophotometry studies of other members of the family Pleuronectidae (*Platichthys flesus* [flounder] and *Pseudopleuronectes americanus* [winter flounder]) show that the peak absorbance of the photopigment in rod cells is ≈510 nm, that in single cones is ≈450 nm, and that in double cones is ≈530 or 550 nm (Evans et al., 1993; Jokela-Määttä et al., 2007). From these lines of evidence, and the results obtained by fitting rhodopsin absorbance templates to the ERG data by the maximum likelihood method (Fig. 4), we concluded that Pacific halibut retinas contain two photopigments with absorbance maxima similar to other members of the family Pleuronectidae. Our conclusion is also in general agreement with the maximum spectral sensitivities of a range of coastal and continental shelf species (Levine and MacNichol, 1979; Bowmaker, 1990).

The change in spectral sensitivity curves of Pacific halibut (Fig. 3) caused by exposure to bright light and the results of photopigment template fitting (Fig. 4) imply that it is the functional properties of a single class of cells (those containing the longer wavelength ≈520 to 540 nm photopigment) which are predominately disrupted by exposure to bright light. The retinas of larval Atlantic halibut and adults of the related species—winter flounder—are dominated by the so-called "green cones" (Evans et al., 1993; Helvik et al., 2001). If this is also the case in Pacific halibut, our results indicate disruption of the photoreceptor cells which normally provide maximal quantal absorption in the green-light dominated coastal waters (Levine and MacNichol, 1979; Lythgoe, 1975, 1980; Crescitelli, 1991). This detriment to vision is likely to have severe consequences for postrelease predator avoidance and foraging success. Moreover, the results from photopigment template fitting indicate that the specific functional deficit persists for at least 10 to 12 hours after the exposure to bright light and worsens with time (Fig. 4). If this diminished functionality is due to apoptosis induced by photic injury (Wu et al., 2006), it is likely that it will continue to worsen progressively and be permanent.

Hook-and-line caught Pacific halibut can be discarded quickly and are not subject to significant time periods out of the water (Kaimmer and Trumble, 1998). Because they would not be exposed to direct sunlight for prolonged periods, minimal or no reduction in visual function would be expected. In contrast, trawl-caught Pacific halibut can be exposed to bright light during prolonged sorting operations (Davis and Olla, 2001). Increased mortality has been demonstrated in Pacific halibut that remain on deck for 20–40 min (Trumble et al., 1995). Although the exact causes are unknown, our results clearly imply that these fish could have had significant visual impairment when discarded. Development of procedures to improve survival of Pacific halibut may therefore require not only reducing sorting time (and therefore reducing exposure to air and extreme temperatures), but also sheltering the fish from bright light.

The low flicker fusion frequency and high light sensitivity of Pacific halibut are characteristics of a visual system adapted to function at low light levels (Warrant, 1999). We hypothesize that these features make Pacific halibut, and other demersal fishes with similarly structured visual systems, more susceptible to damage by exposure to direct sunlight than species normally inhabiting brightly lit environments. Should this be the case, there would be significant implications for fishery management, although obviously many significant questions remain. For example, we do not know how long individuals have to be exposed to direct sunlight to incur deficits in visual acuity, nor do we know the threshold of light intensity that causes retinal damage. Research is also warranted on exactly which of the various cell types within the retina are being damaged, the effect(s) of light-induced visual deficits on predator-avoidance and prey-finding behaviors, the resultant changes in rates of mortality, and possible effects at the population level.

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Changes in a benthic megafaunal community due to disturbance from bottom fishing and the establishment of a fishery closure

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Abstract—Trawling and dredging on Georges Bank (northwest Atlantic Ocean) have altered the cover of colonial epifauna, as surveyed through *in situ* photography. A total of 454 photographs were analyzed from areas with gravel substrate between 1994 and 2000 at depths of 40–50 m and 80–90 m. The cover of hydroids, bushy bryozoans, sponges, and tubeworms was generally higher at sites undisturbed by fishing than at sites classified as disturbed. The magnitude and significance of this effect depended on depth and year. Encrusting bryozoans were the only type of colonial epifauna positively affected by bottom fishing. Species richness of noncolonial epifauna declined with increased bottom fishing, but Simpson's index of diversity typically peaked at intermediate levels of habitat disturbance. Species that were more abundant at undisturbed sites possessed characteristics that made them vulnerable to bottom fishing. These characteristics include emergent growth forms, soft body parts, low motility, use of complex microhabitats, long life spans, slow growth, and larval dispersal over short distances. After the prohibition of bottom fishing at one site, both colonial and noncolonial species increased in abundance. Populations of most taxa took two years or more to increase after the fishing closure. This finding indicates that bottom fishing needs to be reduced to infrequent intervals to sustain the benthic species composition of Georges Bank at a high level of biodiversity and abundance.

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Commercial fishing with mobile gear (e.g., otter trawls, beam trawls, scallop dredges) has become such a widespread practice that it is estimated that an area equivalent to approximately 75% of the world's continental shelves is trawled annually (Kaiser et al., 2002). Because bottom fishing can kill and injure benthic invertebrates that are either caught as bycatch or crushed underneath fishing gear, heavily fished areas often exhibit decreased abundance, biomass, and diversity of epifauna (Collie et al., 1997). Trawling and dredging can modify the composition of marine sediments through the dispersal of piles of boulders (Collie et al., 1997; Freese et al., 1999) and the resuspension of fine sediments. The latter process may subsequently release contaminants and excess nutrients, expose anoxic sediment layers, smother filter feeders, and alter biogeochemical fluxes (Kaiser et al., 2002). Bottom fishing also affects benthic community structure by augmenting the abundance of scavengers (Ramsay et al., 1998) and depleting organisms that serve as ecosystem engineers or keystone species (Auster et al., 1996). Similarly, ecosystem function and population dynamics can be indirectly altered because bottom fishing may reduce levels of epifaunal productivity (Hermsen et al., 2003), lower the diversity of prey available to demersal fish (Jiang and Carbines, 2002), and decrease the structural complexity of the benthic environment (Auster et al., 1996).

A meta-analysis of the effects of bottom fishing revealed that less than one-half of the studies addressing this topic examined the long-term recovery of the benthic community (Collie et al., 2000b). In studies where recovery rates were investigated, most tracked recovery for a period of less than two years, which may not be long enough to evaluate the final outcome of ecological succession in disturbed habitat patches. For example, at the completion of a study that involved experimental trawling in a Scottish sea loch, physical signs of trawl damage had disappeared after 18 months, but differences in the benthic community structure at treatment and reference sites persisted (Tuck et al., 1998). Similarly, at the end of a six-month study of the effects of experimental trawling, the polychaete *Terebellides atlantis* and the nemertean *Cerebratulus lacteus* had not yet returned to pretrawling levels of abundance (Sparks-McConkey and Watling, 2001). Knowledge of epifaunal recovery from natural and fishing-induced disturbance is especially lacking on the continental shelf, an area subjected to heavy bottom fishing. Compared to intertidal zones and shallow subtidal areas where benthic responses to natural disturbance have long been studied, relatively little research has been undertaken to track the colonization of sessile epifauna to hard substrata at depths greater than 30 m after natural or anthropogenic disturbances. In the Gulf of Maine, those studies on this subject (Sebens et al., 1988; Witman, 1998) had relatively short durations (less than two years), which may not have

provided adequate time for the completion of ecological succession.

We evaluated fluctuations in the abundance of colonial and noncolonial epifauna in benthic photographs taken between the years 1994 and 2000 in areas of Georges Bank (northwest Atlantic) that have been classified as either disturbed or undisturbed by bottom fishing. Colonial epifauna are ecologically important because many taxa generate three-dimensional microhabitats that augment the structural complexity of the benthic environment. These microhabitats may directly benefit invertebrates and demersal fishes by aggregating food sources and providing refuge from visual predators (Henkel and Pawlik, 2005). Comparisons were made between two depth strata in order to determine how depth may modulate the response of epifauna to bottom-fishing disturbance. Because noncolonial species of megafauna were examined in both photographs and previously analyzed benthic samples collected at the same study sites with a 1-m wide "naturalist" dredge (a dredge design described in Eleftheriou and Holme, 1984), trends in noncolonial epifauna distribution, abundance, and species composition are analyzed to facilitate comparisons between sampling techniques. The effect of bottom fishing on the diversity of noncolonial species is also examined.

One of our study sites was located in an area where bottom fishing has been prohibited since December 1994. Because this site had previously been fished with mobile gear, much of its resident epifauna had been destroyed at the beginning of our study. This situation allowed us to examine patterns of ecological recovery. Our research represents one of the first published studies conducted in this geographic region on the long-term processes (i.e., greater than two years) affecting ecological recovery from disturbance among colonial epifauna on the continental shelf.

Materials and methods

Description of study sites

In December 1994, bottom fishing was prohibited in three large sections of Georges Bank and the southern New England continental shelf to decrease levels of fishing mortality on Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), and yellowtail flounder (*Limanda ferruginea*). Closed (fishing) area II (CA-II), which is the focus of the current research, is located 150 km offshore on the Northeast Peak of Georges Bank (Fig. 1A). The northern section of CA-II where the seafloor is principally covered by gravel substrate was designated as a habitat area of particular concern (HAPC) in 1998. The gravel substrate in this area forms an important habitat for Atlantic sea scallops (*Placopecten magellanicus*), provides a nursery ground for juvenile *G. morhua* and *M. aeglefinus*, and serves as a spawning ground where Atlantic herring (*Clupea harengus*) deposit their demersal eggs (Lough et al., 1989). Sections of the gravel substrate contain dense patches of sponges, hydroids, bushy and encrusting bryozoans, colonial ascidians, and the lacy tubeworm (*Filograna implexa*) (Collie et al., 2000a; Valentine et al., 2007).

During the year before the establishment of CA-II, scientists from the University of Rhode Island (URI) and the United States Geological Survey (USGS) traveled to this area on two occasions to conduct sidescan sonar surveys, collect samples of benthic megafauna, and record the fauna observed on the seafloor along transect lines by means of video and still cameras. Initial assessments of the benthic megafauna and colonial epifauna at these study sites were published in Collie et al. (1997, 2000a). Between 1994 and 2000, the URI and USGS research team returned to Georges Bank on a nearly annual basis to track the recovery of the megafaunal community in CA-II and to evaluate interannual variations in community structure at other sites (Collie et al., 2005).

Because some sites on Georges Bank contain scattered boulders that can damage fishing gear, certain areas are generally avoided by fishermen and can, therefore, be classified as undisturbed by bottom fishing. To identify which regions were disturbed by bottom fishing, a 10-km^2 area at six different sites was surveyed with a 100-Khz high-resolution sidescan sonar in April 1994. Sonograms from these surveys were inspected for trawl and dredge marks. Areas containing such marks were classified as disturbed, and areas with no visible signs of recent trawling or dredging activity were considered undisturbed. Sediment maps were consulted to ensure that only sites dominated by gravel substrate were sampled (Valentine et al., 1993). Disturbed and undisturbed sites were examined at shallow depths (40–50 m) and at deeper depths (80–90 m) in U.S. and Canadian waters (Collie et al., 1997).

The original classification of study sites as disturbed or undisturbed was validated with data on scallop dredging and bottom trawling effort provided by the National Marine Fisheries Service (NMFS) and the Canadian Department of Fisheries and Oceans (DFO) (Table 1). For sites located within the U.S. Economic Exclusive Zone, data from the NMFS satellite vessel monitoring program were used to compute the number of hours that scallop fishing boats spent dredging each square nautical mile of Georges Bank from 1998 through 2000 (Collie et al., 2005). Before 1998, the classification of sites as disturbed or not disturbed on the U.S. portion of Georges Bank was verified with NMFS data on scallop dredging effort which had a 10-minute latitude resolution. At shallow sites, both NMFS data sets consistently confirmed the accuracy of the original disturbance classifications that had been based on sidescan sonar surveys. The disturbance classifications at Canadian sites were authenticated with data from DFO logbook reports from the commercial scallop fishery that had a 1-minute latitude resolution. Because the number of days fished at deep sites varied both inside individual study sites and between years (Collie et al., 2005), we decided that the classifications of sites located at the

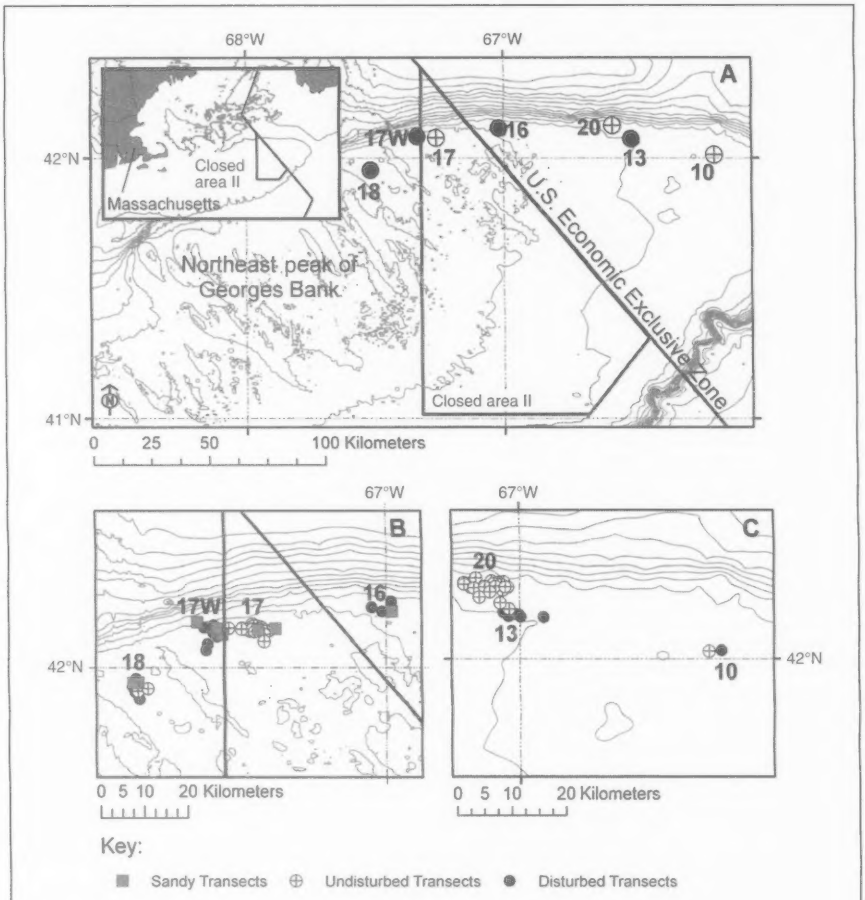

Figure 1

(**A**) Map of the Northeast Peak of Georges Bank displaying closed area II and the study sites surveyed between 1994 and 2000. (**B**) and (**C**) Maps of the starting points of each photographic transect conducted at shallow (B) and deep (C) sites. Transects were classified as sandy if more than 50% of the photographs analyzed contained ≥50% cover of sand. Numbers on each map identify study sites. The classification of disturbance at some sites (i.e., 17 and 18) changed throughout the duration of this study. The lines in the background of each map show the bathymetry of Georges Bank; each line represents a 20-m change in depth.

deeper depth strata should be recategorized. According to our revised classification scheme, any photographed transect at a deep site that was fished with a scallop dredge for one day or less per year was classified as undisturbed.

Based on disturbance classification, sediment composition, and depth, five 5 km × 10 km sites were selected for examination during the first year of this study (Fig. 1). Three of these study sites (i.e., sites 10, 13, and 20) were located in deeper water on the Canadian section of Georges Bank. At the 80–90 m depth stratum, site 20 was classified as undisturbed, site 13 was classified as disturbed for all years except 1998 when it contained two undisturbed transects, and site 10 was sampled only in 1994 when it contained one disturbed and one undisturbed transect (Table 1). On the shallower U.S. portion of the bank, two study sites were originally surveyed, one corresponding to an area with little disturbance from bottom fishing (i.e., site 18) and the other representing a heavily fished zone (i.e., site 17). After the incorporation of site 17 into CA-II, much of the fishing effort formerly concentrated at this site was displaced into areas that were unaffected by the closure, including site 18. Consequently, the initial clas-

Table 1

Classifications of disturbance and mean scallop dredging effort at study sites based on data from the National Marine Fisheries Service (NMFS) and the Canadian Department of Fisheries and Oceans (DFO). Dashes indicate years when no photographic samples were collected. D and U indicate shallow sites that were classified as "disturbed" or "undisturbed," respectively, before the development of the NMFS satellite vessel monitoring program in 1998. Classifications made at shallow sites before 1998 were based on trawl and dredge marks seen in side can sonar images. No photographic data were gathered in 1995. Dredging effort from U.S. and Canadian sites cannot be easily compared because data were reported in different units and the type of fishing gear used by each country may have varied.

Site	Classification of disturbance	1994	1996	1997	1998	1999	2000
Shallow sites: Mean dredging effort in hours fished per year within two miles (3.2 km) of site							
16	Disturbed	—	D	D	—	—	—
17	Disturbed before establishment of a fishery closure (i.e., 1994) and undisturbed afterwards (i.e., 1996–2000)	D	U	U	0	0	0
17W	Disturbed	—	—	D	172.6	58.1	—
18	Undisturbed before establishment of a closed area (i.e., 1994) and disturbed afterwards (i.e., 1996–2000)	U	D	D	97.4	1.0	1.2
Deep sites: Mean dredging effort in days fished per year within a square minute latitude of a transect							
10	Mildly to moderately disturbed	2.5	—	—	—	—	—
13	Disturbed, except for two transects surveyed in 1998	3.5	5.0	4.0	2.0	—	—
20	Undisturbed	0	0.2	0.1	0	0	—

sification of bottom-fishing-induced disturbance at sites 17 and 18 was reversed in 1995. In order to evaluate the effectiveness of the fishery closure, other disturbed sites were sampled surrounding CA-II (i.e., sites 16 and 17W) in later years.

Fieldwork and laboratory procedures

Photographs of the seafloor of northeastern Georges Bank were collected during seven research cruises, which were conducted on a nearly annual basis from November 1994 through November 2000. During all cruises, except for one occurring in July 1995, the SEABed Observation and Sampling System (SEABOSS; Woods Hole Science Center, U.S. Geological Survey, Woods Hole, MA) was deployed to take 35-mm photographs of the seafloor. The SEABOSS consists of a tethered van Veen grab sampler that is equipped with two Hi-8 video cameras, one still camera, accompanying lights, and a pressure-depth sensor (Blackwood and Parolski, 2001). The SEABOSS also contains two parallel lasers placed 20 cm apart, which are used to gauge the size of objects on the seabed. The frame of the SEABOSS instrument was attached to a winch and allowed to drift under the research vessel as it traveled at a speed of 1–2 knots (0.5–1.0 m/s). At each study site surveyed during a research cruise, three transects were generally conducted with the SEABOSS. However, depending on weather conditions, research priorities, and available ship time, anywhere between one and ten transects were filmed. Because of changes in tidal speed, transect length varied between 183 and 4316 m (mean distance of 921 m, standard deviation [SD]=527 m). Along transects, photographs were taken at 30–60 s intervals. In addition to the SEABOSS, during the 1996 and 1999 research cruises, videos and photographs of the seafloor were also taken with a MaxRover MK1 remotely operated vehicle (ROV; Deep Sea Systems International, Inc., Falmouth, MA) provided by the National Undersea Research Center at the University of Connecticut. This ROV system included a set of two 35-mm still cameras and two Hi-8 video cameras. As with the SEABOSS, two parallel lasers placed 20 cm apart were used to estimate the size of objects on the seafloor. During the 30-minute ROV transects (approximately 1000 m in length), photographs of the seabed were snapped at 30-second intervals. Each photograph taken with the SEABOSS covered an area of approximately 0.25–0.27 m^2 of the seafloor, whereas approximately 0.31 m^2 of the sea bottom was examined in each ROV photo.

Because of the impracticality of analyzing the hundreds of photographs taken during each cruise, we randomly selected a subsample of 12–30 photographs per site (average=16) to be examined in detail each year. As a result of the scarcity of quality photos (i.e., not blurry or sandy), a reduced number of photographs were chosen from site 13 in 1996 and site 20 in 1999. A total of 454 benthic photographs from 94 transects surveyed from 1994 and 2000 were analyzed. These photographs covered an area of 120.8 m^2 of the seafloor. Following the method described in Collie et al. (2000a), we over-

laid photographs with a transparency containing a grid, in which each grid cell represented a 5 cm × 5 cm area of the seafloor. From 1996 through 2000, the percent cover of hydroids, bushy and encrusting bryozoans, sponges, and *F. implexa* was recorded in each grid cell. Data were summed across grid cells to calculate the total percent cover of each type of colonial organism per photograph. In 1994, the percent frequency of several types of colonial epifauna was measured, instead of percent cover. Results of the 1994 surveys were presented in Collie et al. (2000a). In addition, noncolonial, megafaunal species were enumerated in each grid cell and identified to the lowest possible taxonomic level. Sediment type and the number of pieces of cultch (i.e., broken bivalve shells) were also recorded during analyses of the photographs. Like colonial epifauna, some fish species use the three-dimensional structure generated by cultch to obtain shelter from predators (Auster et al., 1995).

Because hard bottom (e.g., cobble and gravel) and soft bottom (e.g., sand, silt, and mud) environments support fundamentally different benthic communities, we decided to remove from our data set those transects where sand constituted a large percentage of the substratum. Transects where the majority of photographs contained greater than 50% sand cover were usually clustered around a distinct area or were located at the far edge of our study sites (Fig. 1B). To guarantee that only gravel habitat was examined, 34 photographs from sandy transects were removed from the data set. Once photographs that were sandy, blurry, or overexposed had been selected and removed, 386 photographs remained, covering an area of 100.1 m^2.

Throughout this study, benthic photographs were analyzed by five observers. To evaluate the extent to which between year and within-year fluctuations in epifauna abundance might reflect observer bias, calibration tests were performed during which two observers examined the same photograph(s). *T*-tests, in which photographs analyzed by different observers were treated as matched pairs, were used to determine whether observer bias affected estimates of epifaunal abundance. Results indicated that, with the exception of the coiled worm (*Spirorbis* spp.), patterns of observer bias did not match the direction and magnitude of between-year and within-year variations in epifaunal cover or megafaunal abundance (Asch, 2006). Data on *Spirorbis* spp. are not presented here because of concerns about observer bias.

Statistical analyses

Colonial epifauna A series of two-way analysis of variance (ANOVA) tests were conducted to investigate which taxa of colonial epifauna exhibited significant differences in percent cover between disturbance categories and years. Because large variations in organismal abundance with depth may overshadow subtler fluctuations related to bottom fishing, each depth stratum was considered separately in the ensuing analyses. Response variables included six measures of the cover of colonial epifauna taxa and cultch. A previous examination of spatial patterns indicated that autocorrelation existed between photos from the same transect (Asch, 2006). Therefore, percent cover from photos were averaged across transects, allowing us to use transects as our primary sampling unit when conducting parametric tests. All response variables were arcsine square-root transformed in order to ensure that percent cover data would conform to the normal probability density function. Bottom-fishing disturbance classifications and year were used as factors in the ANOVAs. At shallow sites, the interaction term (*disturbance* × *year*) was used to evaluate whether differences between sites inside and outside of CA-II increased over time because of the continued recovery of organisms in CA-II. Since no data from a deep, disturbed site were collected in 1999, this particular year was removed from the data set when we considered the deeper depth stratum, so that the experimental design would be balanced. Because of the nonorthogonal design of these ANOVAs, type-III sums of squares were used. When interannual differences were indicated to be an important factor affecting a particular type of colonial epifauna, Tukey-Kramer multiple comparisons tests were applied to determine which years differed significantly (S-Plus 6, MathSoft, Inc., Seattle, WA).

Noncolonial organisms Although many noncolonial organisms were identified to the species level, other organisms could only be identified to genus or family because of the limited resolution of photos or the need for microscopic examination of distinguishing features. In cases where some organisms belonging to a particular taxonomic group could be identified to species, but others could not, all members of the taxonomic group in question were lumped together in order to guarantee that classifications were mutually exclusive. Ninety-eight percent of the noncolonial organisms identified in photographs belonged to five extremely abundant taxa: *Spirorbis* spp., the tubeworm *Protula tubularia*, the jingle shells *Anomia* spp., the barnacles *Balanus* spp., and an unidentified species of burrowing anemones (order Ceriantharia). For each depth and disturbance category, the R statistical package (Free Software Foundation, Boston, MA) was used to fit the negative binomial distribution to the data on the abundance of these five species. Next, a *U* test was applied to evaluate the goodness-of-fit of the negative binomial distribution (Krebs, 1999). Because in all cases but one (i.e., *Anomia* spp. at deep, undisturbed sites) the negative binomial distribution provided an adequate fit, we determined that these five highly abundant species have an aggregated spatial distribution, whereby they were absent from many photographs but obtained very high densities in a few areas. For example, *Anomia* spp. and *Balanus* spp. were not identified in two-thirds of the photographs sampled but were occasionally observed at concentrations as high as 1328 per m^2 and 760 per m^2, respectively. Because random sampling of such dense aggregations could result in the detection of artificial

differences among depths, disturbance categories, and years, data on the abundance of these five species are not presented in this manuscript. However, information on trends in the abundance of these five taxa can be referenced in Asch (2006).

All other noncolonial taxa were collectively examined as a multivariate data set. Differences in the species composition of these noncolonial organisms among depth strata, years, bottom-fishing disturbance categories, and sites were explored by constructing nonmetric multidimensional scaling (MDS) plots in PRIMER 6 (Plymouth Marine Lab, Plymouth, UK). MDS plots were constructed with a Bray-Curtis similarity matrix containing information on the mean number of individuals of each taxon per photograph, aggregated across each transect (Clarke and Warwick, 2001). To ensure that patterns in the MDS plot were not dictated solely by trends in the abundance of a few ubiquitous species, data were square-root transformed before this analysis and all other multivariate statistical routines (i.e., analysis of similarity [ANOSIM] and similarity of percentages [SIMPER]). ANOSIM tests were used to evaluate whether differences in noncolonial species composition between disturbance categories and years were statistically significant (Clarke and Warwick, 2001). In cases where significant differences were detected, the SIMPER routine in PRIMER 6 was used to determine the mean percent dissimilarity that each species contributed to differences between disturbance categories (Clarke and Warwick, 2001).

In addition to examining the effect of bottom-fishing-induced disturbance on species composition, its influence on two measures of noncolonial diversity was investigated. Jackknife species richness and Simpson's index of diversity ($1-\lambda'$) were calculated according to the methods described in Krebs (1999). These two diversity indices were selected, because 1) they are either unaffected by differences in sample size (e.g., Simpson's index) or incorporate estimates of the number of rare species that are unlikely to be sampled (e.g., jackknife species richness) (Clarke and Warwick, 2001), and 2) together these indices measure several aspects of biodiversity (e.g., richness, heterogeneity, and evenness). The diversity indices were computed for five levels of disturbance at both shallow and deep sites. Data were binned into five disturbance levels to achieve sufficient sample sizes because the jackknife species richness index requires that the size of samples is large enough to include at least half of the species in an area (Krebs, 1999). Only data from shallow sites collected during years when the NMFS satellite vessel monitoring program was in operation (i.e., 1998–2000) were analyzed. Linear and polynomial regressions were performed to evaluate the relationship between bottom-fishing disturbance and these two measures of noncolonial diversity. The most parsimonious regression model was selected on the basis of the results of the analysis of deviance test included in the R statistical package.

Results

Colonial epifauna

At shallow sites, the cover of five out of six taxa of colonial epifauna differed significantly between undisturbed and disturbed sites located, respectively, inside and outside of CA-II (Fig. 2). Hydroids were the only taxon of colonial epifauna whose percent cover was not significantly affected by disturbance at shallow sites. During most years, structurally complex taxa of colonial epifauna, such as sponges and bushy bryozoans, were more abundant at shallow, undisturbed sites, whereas encrusting taxa, such as encrusting bryozoa, cultch, and *F. implexa*, exhibited a higher percent cover at shallow, disturbed sites (Fig. 2). With the exception of *F. implexa* whose cover at each shallow site remained fairly stable throughout the time series, all other colonial epifauna taxa demonstrated significant between-year variations in abundance at shallow sites. Several of these taxa showed marked changes in abundance beginning in 1997 and 1998, indicating that it took at least two years for these species to respond to the establishment of CA-II or to the increased bottom-fishing effort at sites that remained open to mobile fishing gear. For example, sponges were absent from all photographs taken at the shallow site in CA-II during 1994 and 1996 but were seen in 97% of the photographs taken in this area in 1997 (Figs. 2 and 3A). Similarly, bushy bryozoans experienced a temporary spike in their percent cover at the shallow, undisturbed site in 1997. The cover of encrusting bryozoans was fairly similar at all shallow sites at the initiation of this study, but then began to increase in disturbed areas beginning in 1998. By the year 2000, 62% of the substratum at shallow, disturbed sites was covered by encrusting bryozoa (Fig. 3B). Temporal variations in the abundance of hydroids and cultch are unlikely to reflect changes in bottom-fishing effort because similar between-year changes were seen at all shallow sites regardless of closure status.

The only two taxa that clearly exhibited significant differences between disturbed and undisturbed sites at the deep depth stratum were *F. implexa* and hydroids, both of which consistently had an elevated percent cover in undisturbed areas (Fig. 4). Bushy bryozoans also had a higher cover in 1997 and 1998 in deep areas that experienced little to no bottom fishing. However, this pattern was reversed in 1996 when deep, disturbed sites exhibited slightly greater cover of bushy bryozoans than deep, undisturbed sites. Cultch and encrusting bryozoans at deep sites were frequently more abundant in disturbed areas, but this trend was inconsistent between years, causing ANOVA results to be only marginally significant (i.e., $P=0.08$ and $P=0.07$ for cultch and encrusting bryozoans, respectively). Sponge abundance was depressed at all deep sites from 1996 through 1998, and its mean percent cover never exceeded 0.5% during these years. Temporal variations in colonial epifauna cover appeared to be of lesser importance at deep sites than at shallow sites because encrusting bryozoans were

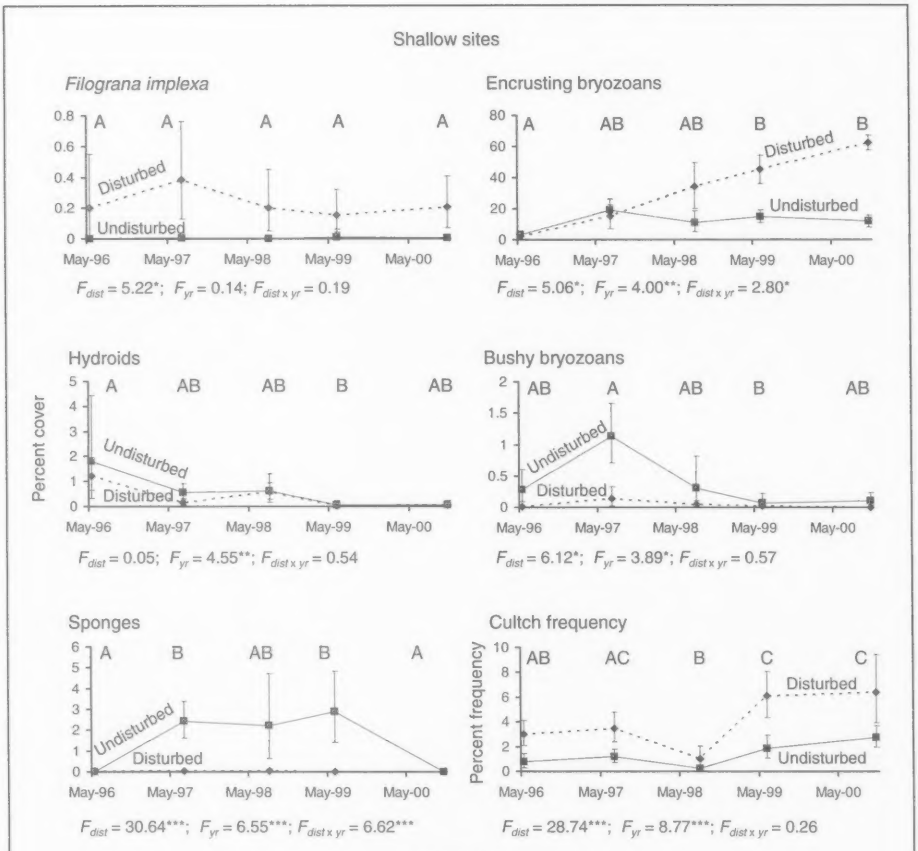

Figure 2

Temporal trends in colonial epifaunal percent cover and cultch percent frequency at shallow sites (40–50 m). Trend lines from sites that were either disturbed or undisturbed by bottom fishing are shown. Shallow undisturbed data came from site 17, whereas shallow disturbed data were derived from sites 16, 17W, and 18. The scale of the *y* axis varies between graphs of different epifaunal taxa. Markers (i.e., black squares and black diamonds) are back-transformed means, and error bars are 95% confidence intervals. Different letters above data points represent annual means that differ significantly from each other in multiple comparison tests. *F* statistics from two-way analyses of variance (ANOVAs) on the effects of disturbance (*dist*), year (*yr*), and disturbance × year (*dist* × *yr*) are shown below each graph (df=1,4,4). Significance levels from ANOVA tests are indicated as follows: **P*<0.05; ***P* <0.01; ****P*<0.001.

the only taxon to have a significant year term in the two-way ANOVAs performed on deep sites (Fig. 4).

The greater between-year variability at shallow sites can be interpreted as a sign of the effectiveness of CA-II. Because there can be subtle, pre-existing differences in habitat quality between areas inside and outside a marine reserve, a conclusive demonstration of reserve effectiveness typically requires that biological distinctions between fished and unfished areas become amplified over time (Murawski et al., 2004). As a result, variables truly affected by the establishment of CA-II should have significant interaction terms in their corresponding two-way ANOVA, as well as exhibit significant differences between disturbance categories and among years. Sponges and encrusting bryozoans were the two colonial epifauna taxa that exhibited significant interaction terms in the ANOVAs performed for shallow sites. No interactions between disturbance and year were detected at the deep sites. This finding reflects the fact that bottom-fishing effort was fairly consistent over time at deep sites (Table 1; Collie et al., 2005), whereas fishing effort at shallow sites showed a more distinct temporal trajectory related to the establishment of CA-II.

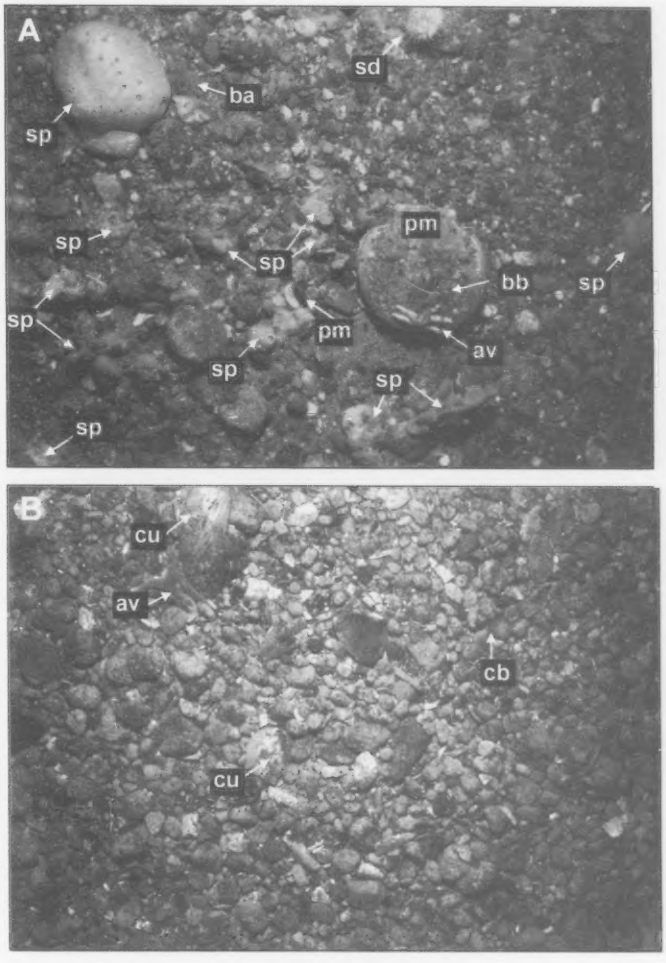

Figure 3

Photographs of the seafloor taken in 1999 at sites 17 (**A**) and 18 (**B**). (A) Sponges (sp) cover 10.4% of the seafloor in this photograph, and bushy bryozoans (bb) cover an additional 2.5%. Species of noncolonial epifauna seen in this photograph include the sea scallop (*Placopecten magellanicus* [pm]), the Northern seastar (*Asterias vulgaris* [av]), the green sea urchin (*Strongylocentrotus droebachiensis* [sd]), and a burrowing anemone (ba). (B) Encrusting bryozoans cover 74.2% of the seafloor in this photo from a shallow, disturbed site. Areas where the gravel substrate is a light grey color contain encrusting bryozoans. Two pieces of cultch (cu) are visible, as well as *A. vulgaris* (av) and a northern cyclocardia (*Cyclocardia borealis* [cb]). Photographs by Dann Blackwood, Woods Hole Science Center, U.S. Geological Survey, Woods Hole, MA.

Noncolonial organisms

In the 454 benthic photographs that were analyzed, a total of 117,315 noncolonial organisms were identified, which included 73 distinct taxa. After removing data on five abundant species with extremely aggregated spatial distributions, we found that this data set contained 1561 individuals. Clear differences between deep and

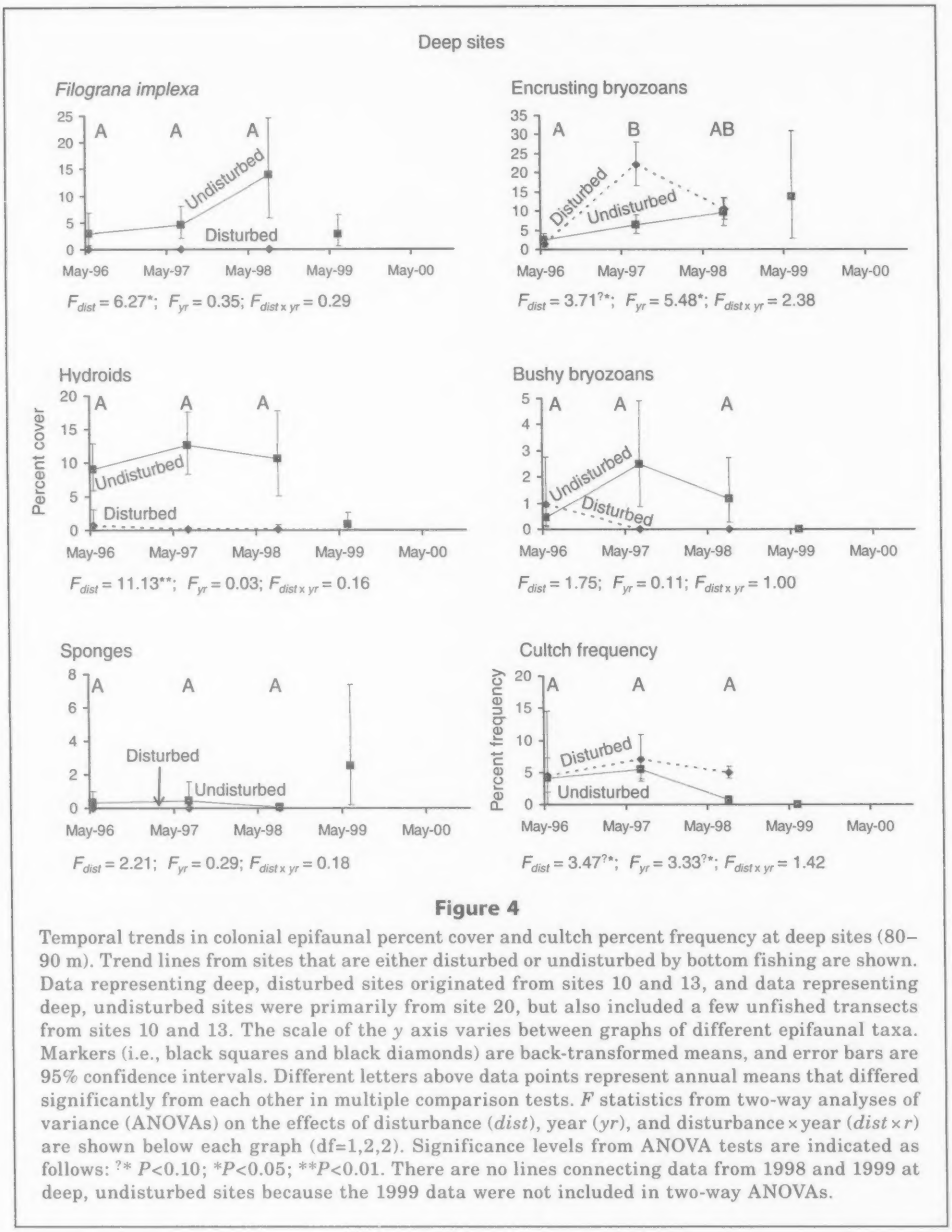

Figure 4

Temporal trends in colonial epifaunal percent cover and cultch percent frequency at deep sites (80–90 m). Trend lines from sites that are either disturbed or undisturbed by bottom fishing are shown. Data representing deep, disturbed sites originated from sites 10 and 13, and data representing deep, undisturbed sites were primarily from site 20, but also included a few unfished transects from sites 10 and 13. The scale of the *y* axis varies between graphs of different epifaunal taxa. Markers (i.e., black squares and black diamonds) are back-transformed means, and error bars are 95% confidence intervals. Different letters above data points represent annual means that differed significantly from each other in multiple comparison tests. *F* statistics from two-way analyses of variance (ANOVAs) on the effects of disturbance (*dist*), year (*yr*), and disturbance × year (*dist* × *r*) are shown below each graph (df=1,2,2). Significance levels from ANOVA tests are indicated as follows: ?* $P<0.10$; *$P<0.05$; **$P<0.01$. There are no lines connecting data from 1998 and 1999 at deep, undisturbed sites because the 1999 data were not included in two-way ANOVAs.

shallow transects in terms of noncolonial megafaunal abundance were apparent when we examined MDS plots (Fig. 5A). Compared to the evident effect of depth on noncolonial species composition, the influence of bottom-fishing disturbance in MDS plots was subtle. Transects recorded at disturbed site 18 could be distinguished from transects at all other shallow sites. Relatively few differences in noncolonial species composition could be seen among sites 16, 17, and 17W (Fig. 5B). At the deep depth stratum, disturbed sites 10 and 13 generally exhibited a community composition that was distinct from undisturbed site 20, as indicated by the positioning of disturbed transects to the left of the MDS plot (Fig. 5C). The two transects from site 13 that were more interspersed among transects from site 20 in the MDS were filmed in the northwestern extent of this study site, which was subjected to less bottom-fishing pressure. The greater dispersion of deep, undisturbed transects across the MDS revealed a wider variation in species composition at site 20 than at other deep sites.

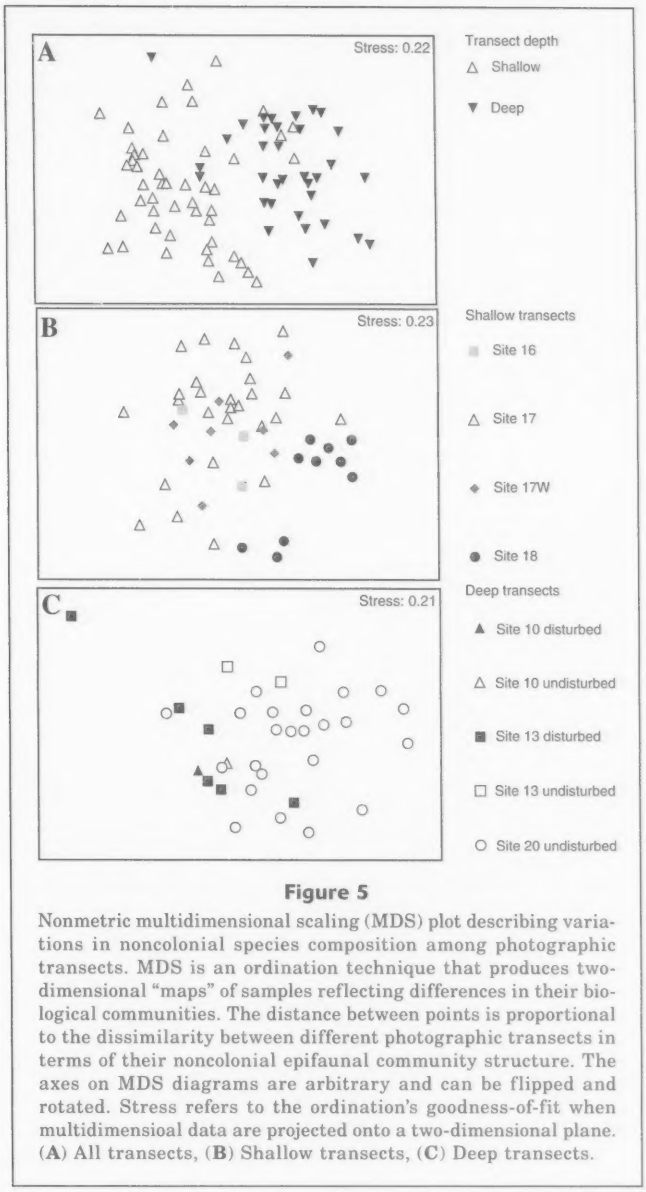

Figure 5

Nonmetric multidimensional scaling (MDS) plot describing variations in noncolonial species composition among photographic transects. MDS is an ordination technique that produces two-dimensional "maps" of samples reflecting differences in their biological communities. The distance between points is proportional to the dissimilarity between different photographic transects in terms of their noncolonial epifaunal community structure. The axes on MDS diagrams are arbitrary and can be flipped and rotated. Stress refers to the ordination's goodness-of-fit when multidimensioal data are projected onto a two-dimensional plane. (**A**) All transects, (**B**) Shallow transects, (**C**) Deep transects.

The majority of noncolonial species were most abundant at undisturbed sites, but hard-shelled mollusks and scavengers tended to dominate in heavily fished areas. Significant variations in community structure were detected at both depths between different disturbance levels and years by means of ANOSIM tests (Tables 2A and 3A). A total of six taxa contributed to 50% of the cumulative dissimilarity between disturbed and undisturbed sites at the shallow depth stratum (Table 2B). Of these six taxa, *P. magellanicus*, the green sea urchin (*Strongylocentrotus droebachiensis*), and the hermit crabs *Pagurus* spp. exhibited higher levels of abundance at shallow sites inside CA-II. Scavenging seastars (*Asterias* spp.) and bivalves (astartes *Astarte* spp. and the northern cyclocardia *Cyclocardia borealis*) were more abundant along shallow, disturbed transects recorded outside the closed area. The elevated numbers of the latter two species at site 18, as well as siphons from an unidentified, infaunal bivalve, explained why transects from site 18 were separated from other shallow sites in the MDS (Fig. 5B). Of the four species that defined 88% of the similarity among undisturbed

Table 2

(**A**) Effects of disturbance from mobile fishing gear and year on the composition of noncolonial organisms at shallow sites. In this two-way crossed analysis of similarity (ANOSIM) test, the sample global R is the test statistic. Data were permuted 9999 times to generate an empirical frequency distribution for global R. Significance levels: $^{*}P<0.05$; $^{***}P<0.001$. (**B**) Similarity of percentages (SIMPER) results identifying species that are characteristic of disturbed and undisturbed sites at the shallow depth stratum. Cumulative percentage refers to the contribution that species make to either the cumulative similarity within each group or the cumulative dissimilarity between two groups. Results are presented in terms of similarity among disturbed transects, similarity among undisturbed transects, and dissimilarity between disturbance groups.

A ANOSIM results

Factor	95th percentile of permuted global R's	Sample global R
Disturbance	0.14	0.20*
Year	0.12	0.30***

B SIMPER results

Similarity among disturbed transects (i.e., outside CA-II)

Scientific name	Mean density per photograph	Cumulative percentage
Pagurus spp.	0.40	27.55
Asterias spp.	0.32	48.41
Placopecten magellanicus	0.59	63.17
Siphons of unidentified bivalves	0.51	74.24
Cyclocardia borealis	0.24	82.70

Similarity amongst undisturbed transects (i.e., inside CA-II)

Scientific name	Mean density per photograph	Cumulative percentage
Placopecten magellanicus	0.72	43.58
Pagurus spp.	0.42	67.24
Strongylocentrotus droebachiensis	0.30	78.10
Asterias spp.	0.27	88.39

Dissimilarity between disturbance groups

Scientific name	Mean density at disturbed sites	Mean density at undisturbed sites	Cumulative percentage
Placopecten magellanicus	0.59	0.72	12.68
Siphons of unidentified bivalves	0.51	0.09	21.74
Pagurus spp.	0.40	0.42	30.46
Asterias spp.	0.32	0.27	39.07
Strongylocentrotus droebachiensis	0.04	0.30	46.32
Astarte spp.	0.47	0.06	52.49

transects inside CA-II (i.e., *P. magellanicus*, *Pagurus* spp., *S. droebachiensis*, and *Asterias* spp., Table 2B), all increased in abundance from 1994 through 2000 (Figs. 3A and 6). Of particular note was the nearly tenfold increase in the mean density of *P. magellanicus* during this time period. At deep sites, seven of the ten taxonomic groups that contributed the most to dissimilarity among disturbance categories were more abundant along undisturbed transects (Table 3B). As was the case at shallow sites, hard-shelled mollusks (*Astarte* spp. and the waved whelk [*Buccinum undatum*]) and scavengers, such as *Pagurus* spp., characterized deep, disturbed sites.

At both depths, species richness of noncolonial epifauna peaked along undisturbed transects (Fig. 7). The negative relationship between species richness and bot-

Table 3

(**A**) Effects of disturbance from mobile fishing gear and year on the composition of noncolonial organisms at deep sites. In this two-way crossed analysis of similarity (ANOSIM) test, the sample global R is the test statistic. Data were permuted 9999 times to generate an empirical frequency distribution for global R. Significance levels: $^{*}P<0.05$; $^{***}P<0.001$. (**B**) Similarity of percentages (SIMPER) results identifying species that are characteristic of disturbed and undisturbed sites at the deep depth stratum. Cumulative percentage refers to the contribution that species make to the cumulative similarity within each group or the cumulative dissimilarity between these groups. Results are presented in terms of similarity among disturbed transects, similarity among undisturbed transects, and dissimilarity between disturbance groups.

A ANOSIM results

Factor	95th percentile of permuted global R's	Sample global R
Disturbance	0.25	0.28*
Year	0.11	0.41***

B SIMPER results

Similarity amongst disturbed transects

Scientific name	Mean density per photograph	Cumulative percentage
Astarte spp.	0.55	21.75
Pagurus spp.	0.55	42.10
Caridea unident.	1.03	59.65
Gastropoda unident. (shelled)	0.48	70.37
Zoantharia unident.	0.23	79.04
Placopecten magellanicus	0.15	86.93

Similarity amongst undisturbed transects

Scientific name	Mean density per photograph	Cumulative percentage
Zoantharia unident.	1.74	35.80
Astarte spp.	0.64	47.03
Pagurus spp.	0.44	55.14
Caridea unident.	3.52	62.44
Modiolus modiolus	0.53	69.59
Gastropoda unident. (shelled)	0.39	76.16
Hyas coarctatus	0.33	81.47

Dissimilarity between disturbance groups

Scientific name	Mean density at disturbed sites	Mean density at undisturbed sites	Cumulative percentage
Zoantharia unident.	0.23	1.74	11.32
Caridea unident.	1.03	3.52	20.25
Astarte spp.	0.55	0.64	26.33
Modiolus modiolus	0	0.53	32.24
Thelepus cincinnatus	1.14	0.25	38.12
Pagurus spp.	0.55	0.44	43.90
Gastropoda unident. (shelled)	0.48	0.39	49.52
Hyas coarctatus	0.05	0.33	53.97
Ophiuroidea unident.	0.05	0.75	58.34
Polychaeta unident. (tubicolous)	0.29	0.33	62.68

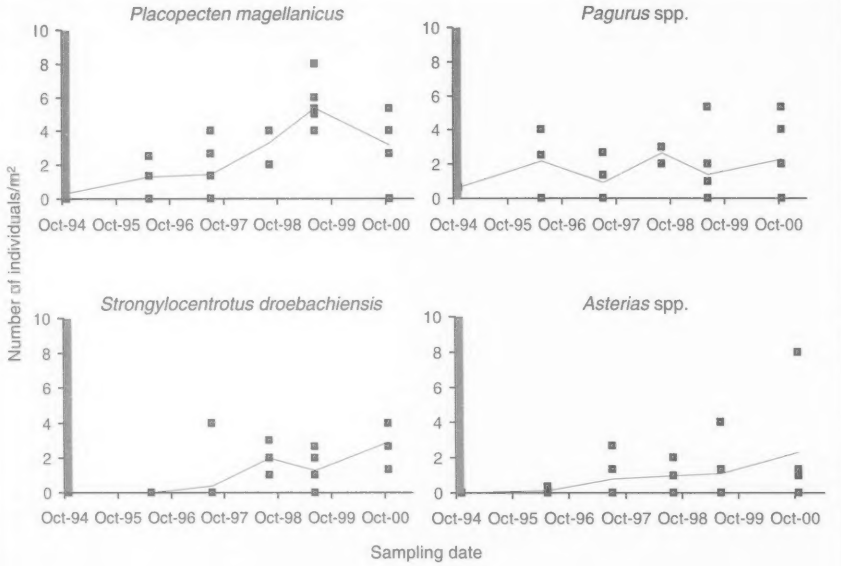

Figure 6

Trends in the abundance of four noncolonial species characteristic of site 17 in closed area II (CA-II). Each black square represents a single photographic transect, and solid lines connect the means from each sampling date. The shaded area indicates the time period before bottom fishing was restricted in CA-II.

tom-fishing effort was statistically significant at shallow sites. Average species richness was greater at deep sites than at shallow sites ($\hat{S}_{deep}$=22 and $\hat{S}_{shallow}$=13), but these two depths did not differ in terms of mean values of Simpson's index of diversity ($1-\lambda'_{deep}$=0.77 and $1-\lambda'_{shallow}$=0.76). The highest values of Simpson's index at shallow sites were recorded in areas with 50–100 hours of scallop dredging per year. At greater levels of bottom-fishing disturbance, a decline in Simpson's index was observed. At deep sites, Simpson's index varied relatively little with disturbance, although its peak value occurred in an area with an intermediate amount of disturbance (i.e., three days of dredging per year). Trends in Simpson's index were not significant when modeled with linear and polynomial regression.

Discussion

Colonial epifauna

Because sessile, colonial organisms cannot avoid the path of mobile fishing gear nor can they quickly immigrate into recently disturbed areas, they may be more adversely affected by bottom fishing than motile species. All six taxa of colonial epifauna examined in this study were affected by bottom fishing, although the magnitude and direction of this effect often depended on depth and varied between years. Because the effect of disturbance on noncolonial species composition was fairly subtle, our study indicates a greater sensitivity of colonial epifauna to disturbance by mobile fishing gear. Similarly, studies conducted in the Gulf of Alaska and Irish Sea show that most motile organisms are less severely affected by chronic and experimental trawling than are anthozoans, sponges, bryozoans, hydroids, tubicolous polychaetes, and barnacles (Freese et al., 1999; Bradshaw et al., 2002). Nevertheless, sessile colonial epifauna exhibit variable morphological forms and possess diverse life history characteristics that can result in different responses to bottom fishing. Colonial organisms whose physical structure can be described as branching, sheet-forming, or mound-forming generally grow slowly but are better competitors for space on the seafloor than encrusting and stoloniferous epifauna (Hughes, 1989). Because of their slow growth patterns, organisms with branching, sheet-forming, and mound-forming structures are typically adapted to stable environments where they are less likely to be detached from their substrate by physical forces associated with natural disturbances. Anthropogenic disturbance causes formerly stable environments to become unstable, thus leaving colonial epifauna with a branching structure maladapted to their new environment.

On Georges Bank, sponges appear to be the colonial epifaunal taxa most negatively affected by bottom fish-

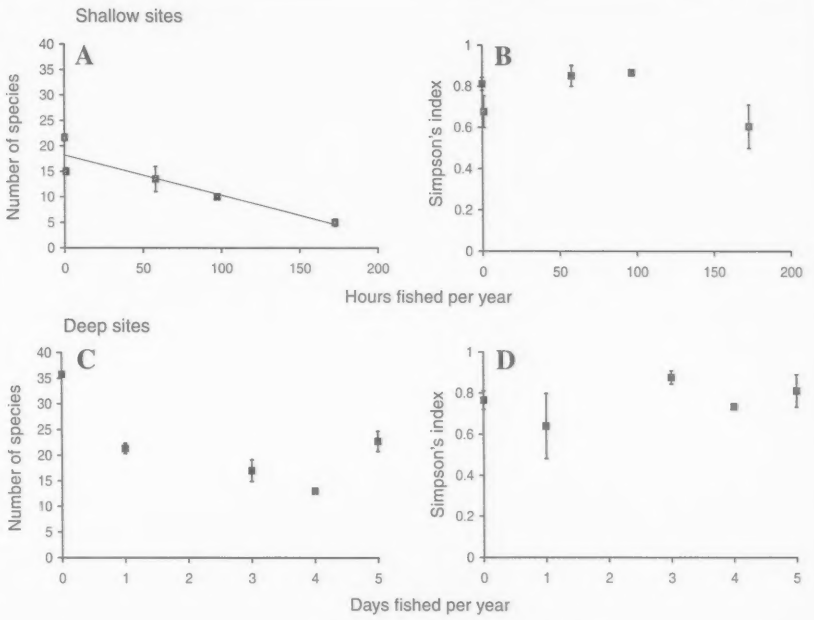

Figure 7

Species richness (number of species) and Simpson's index of diversity ($1-\lambda'$) for noncolonial epifauna at shallow and deep sites. Black squares indicate mean species richness or diversity for a given level of disturbance; standard error bars are shown. Different units of measurement are plotted on the x axis for shallow and deep sites, reflecting the fact that fishing effort was estimated by different methods in U.S. and Canadian waters. Results of linear regression for species richness versus disturbance at shallow sites (**A**): F=17.69; df=1,3; P=0.02. All other linear and polynomial regressions were not significant.

ing at shallow depths. Most sponges at our study sites had branched or mounding-forming structures, which contributed to their sensitivity to mobile fishing gear. Other characteristics that make sponges vulnerable to mobile fishing gear are aperiodic recruitment and a perennial life cycle that allows colonies to persist over several years (Hughes, 1989). In the southern portion of CA-II that is dominated by sandy substrate, sponges are one of the microhabitats whose abundance differs significantly between fished and unfished areas (Lindholm et al., 2004) as was also recorded in our study. Several studies from other regions have documented that sponges are among the most sensitive phyla to bottom fishing (Van Dolah et al., 1987; Freese et al., 1999).

Arborescent epifauna react inconsistently to bottom fishing, reflecting the fact that these organisms possess life history traits that both facilitate and hinder their recovery from physical disturbances. Past research indicates that the effect of bottom fishing on arborescent epifauna varies between regions and species (Auster et al., 1996; Bradshaw et al., 2002). Like sponges, hydroids and bushy bryozoans have an emergent growth form that makes them vulnerable to being knocked over by trawls and dredges. Yet these types of arborescent epifauna do not generally grow as tall as sponges, thus providing them with a degree of resistance to disturbance. When mobile fishing gear do succeed in removing arborescent epifauna from their substrate, recolonization may occur quickly because these organisms are known for rapid growth and typically have short life spans, ranging in length from ten days to one year (Boero, 1983; Hughes, 1989). Other species of hydroids and erect bryozoans are perennial but exhibit seasonal regression. Despite the swift turnover of hydroid and bushy bryozoan colonies, the spatial extent of recovery may be restricted because many species have limited larval dispersal (i.e., <50 m) or tend to settle close to parent colonies (Hughes, 1989; Bradshaw et al., 2002). The dispersal potential of other species, such as hydroids in the family Sertularidae, is even more constrained, because they do not have a pelagic medusa stage (Boero, 1983). Our study indicates that depth may be an additional factor affecting how hydroids react to disturbance caused by bottom fishing, because deeper

sites display a more pronounced, negative response to increased fishing effort. Depth has also been shown to influence the abundance and biomass of other benthic taxa (i.e., amphipods and the brittle star *Ophiura robusta*) in the southern portion of CA-II (Link et al., 2005).

Species in the class Polychaeta play diverse ecological roles (e.g., carnivores, deposit feeders, suspension feeders), use different habitats (e.g., infauna, epifauna), and display different mobility patterns (e.g., errant and tubicolous lifestyles), all of which influences how they respond to bottom fishing. Many polychaetes exhibit a high intrinsic rate of growth allowing them to quickly colonize barren substrate and recover rapidly from disturbances. This high rate of growth explains why the free-living polychaetes in the North Sea remain unaffected by bottom fishing at trawling frequencies as high as six times per year (Jennings et al., 2002). In contrast, tubicolous polychaetes are often heavily affected by bottom fishing because of both their production of calcareous and sediment-encrusted tubes that can be crushed by fishing gear and their need for stable substrate on which to build tubes. This appears to be the case at the deep depth stratum on Georges Bank, where *F. implexa* was less abundant at disturbed sites. Similarly, the inception of bottom fishing was implicated in the declining abundance of four species of tubicolous serpulid polychaetes in the Irish Sea (Bradshaw et al., 2002) and the sabellid polychaete *Myxicola infundibulum* on Fippennies Ledge in the Gulf of Maine (Langton and Robinson, 1990). A few species do prove that there are exceptions to the rule that most tubicolous polychaetes are negatively affected by mobile fishing gear. Such examples include the tubeheads formed by the serpulid polychaete *Pomatoceros* spp. that were not significantly affected by biannual beam trawling in the eastern Irish Sea (Kaiser et al., 1999) and the *Spirorbis* spp. tubes whose abundance was elevated at fished sites on Georges Bank (Collie et al., 2000a; Asch, 2006). The small size of *Spirorbis* spp. tubes makes them extremely difficult to remove from their substrate and may provide this species with a competitive advantage over other species more sensitive to bottom fishing (Collie et al., 2000a). Similarly, the resistance of the *Pomatoceros* spp. to bottom fishing may be related to the fact their tubes are small enough to pass through the 80-mm mesh used by Kaiser et al. (1999) during experimental trawling.

Because of their low, encrusting growth form, encrusting bryozoans have proven to be resistant to physical disturbances from natural sources (Sebens et al., 1988) and appear to be less sensitive to anthropogenic disturbances, as well. The cover of encrusting bryozoans is generally greatest in disturbed areas of Georges Bank. This effect is significant at shallow sites and marginally significant at deep sites. Encrusting bryozoans whose substrate may be overturned by a trawl or dredge are capable of recovering quickly because of their fast growth rates and rapid ability to repair structural damage (Bradshaw et al., 2002). This set of life history characteristics may provide encrusting bryozoans with a competitive advantage in highly disturbed environments.

The abundance of cultch in disturbed areas of northeast Georges Bank can be explained by the fact that most cultch at our study sites consists of *P. magellanicus* shells that were either discarded by scallop fishing crews or killed by mobile fishing gear, but not landed. In areas where cultch accumulation is linked to high levels of predation on bivalves or to current patterns concentrating shell fragments, bottom fishing may have a negative effect on this resource. Such is the case in studies of the effect of mobile fishing gear on patches of cultch on Stellwagen Bank and in a more southern area of Georges Bank (Auster et al., 1996; Lindholm et al., 2004).

Noncolonial organisms

As part of a related research project, naturalist dredge samples of noncolonial megafauna have been collected since 1994 at the same study sites where benthic photographs in the present study were taken (Collie et al., 2005), allowing for comparisons to be made between these two sampling techniques. The results of the current study are similar to those obtained by Collie et al. (2005) in that they both indicated that *P. magellanicus*, *S. droebachiensis*, and *Pagurus* spp. increased in abundance inside CA-II. The increased density of *P. magellanicus* in the closed area is likely related to both direct and indirect effects of bottom fishing. As a commercially targeted species, *P. magellanicus* is removed from areas where scallop dredging occurs. The adverse effect of dredging and otter trawling on sponge cover may also serve to reduce *P. magellanicus* abundance, because some scallop species maintain a mutualistic relationship with sponges that helps them escape predation. In a laboratory experiment where this mutualistic relationship was examined, scallops with sponges encrusted on their shells had to exert 20–30 times less effort to overcome the adherence of the tubefeet of a seastar predator than did scallops whose shells were cleaned of sponges (Bloom, 1975). A third factor influencing the distribution of *P. magellanicus* may be that the seastars (*Asterias* spp.), which are key scallop predators, obtained slightly higher abundance outside the closed area. *Asterias* spp. may be particularly abundant at disturbed sites because members of this genus are also scavengers that have been reported to feed upon organisms damaged by trawls (Ramsay et al., 1998).

The sea urchin *S. droebachiensis* may have also benefited from the elevated cover of colonial epifauna in CA-II because this species is known to eat sponges, hydroids, bryozoans, tunicates, and amphipod and polychaete tube complexes at locations where macroalgae and kelp (the preferred diet of sea urchins) are absent (Briscoe and Sebens, 1988). The elevated number of *S. droebachiensis* in photos taken in CA-II corroborates the findings of Hermsen et al. (2003), who identified

this species as one of the taxa most responsible for increased megafaunal production in CA-II. Along shallow, disturbed transects outside CA-II, bivalves, such as *Astarte* spp. and *C. borealis*, were more abundant. The hard shells of these two species likely confer some resistance to disturbance by bottom fishing. Similarly, the unidentified infaunal bivalve species (most likely the razor clam *Ensis directus*) whose siphons were frequently seen along transects open to fishing may be less vulnerable to this form of disturbance because it resides deep in the substrate below depths affected by otter trawls.

Many of the species most commonly observed at deep, undisturbed sites belong to the classes Anthozoa, Malacostraca, and Ophiuroidea, which are the classes that a meta-analysis has identified as the taxonomic groups most adversely affected by mobile fishing gear (Collie et al., 2000b). At deep sites, we and Collie et al. (2005) found that bottom fishing results in reduced abundance of multiple species of shrimp, brittle stars (e.g., *Ophiopholis aculeata*), tubicolous polychaetes (e.g., *Potamilla neglecta*), and the toad crab *Hyas coarctatus*. Another species that helps define the dissimilarity between disturbed and undisturbed areas at deep sites is the horse mussel *Modiolus modiolus*, which is a long-lived, thin-shelled bivalve that is known to be sensitive to bottom fishing (Bradshaw et al., 2002; Collie et al., 2005). *M. modiolus* may play an important role in the ecology of Georges Bank because anecdotal information from fishermen indicates that the fishes *G. morhua* and *M. aeglefinus* congregate around their beds (Leach, 1998). As was the case at shallow sites, hard-shelled mollusks (i.e., *Astarte* spp. and *B. undatum*) and scavengers, such as *Pagurus* spp., are among the species characteristic of deep, disturbed sites. Both *Pagurus* spp. and *B. undatum* readily consume organisms injured by bottom fishing (Ramsay et al., 1998).

The exact form of the relationship between bottom-fishing disturbance and the diversity of noncolonial species is highly dependent upon the particular diversity index under consideration. At both depth strata, increased bottom fishing results in a concurrent decrease in noncolonial species richness. However, Simpson's index tends to exhibit its highest value at intermediate levels of bottom fishing. This latter pattern is consistent with the intermediate disturbance hypothesis, which proposes that a moderate amount of disturbance can augment diversity by creating more heterogeneous habitats and reducing the likelihood that a single climax species will dominate an area (Connell, 1978). Because bottom fishing is not boosting the absolute number of species present, as indicated by species richness trends, then the high value of Simpson's index at intermediate disturbance levels must be due to a commensurate increase in species evenness resulting from the reduced dominance of a few abundant species. A similar pattern was identified in a study of the effect of scallop dredging on the benthic community of the Irish Sea (Bradshaw et al., 2002).

Recovery period and recovery rates after disturbance

Time periods associated with the recovery of benthic organisms after disturbance caused by bottom fishing are quite variable. Because of adaptations to high levels of natural disturbance, communities living in unconsolidated sand have been predicted to recover from bottom fishing in as little as 100 days (Collie et al., 2000b). However, recovery in structurally complex habitats and among particularly vulnerable species (i.e., species that are long lived, poorly adapted to withstand frequent natural disturbances, or highly susceptible to capture or removal by mobile fishing gear) may require longer time periods. For example, it is estimated to take at least 15 years for several species of sponges off the northwest shelf of Australia to grow to a height of 25 cm (Sainsbury et al., 1997). Similarly, the fig sponge (*Suberites ficus*) became more abundant in areas protected from bottom fishing within a period of 4.5 years (Lindholm et al., 2004). Deep sea corals, such as the samples of *Desmophyllum cristagalli* caught off Western Ireland and calculated to be 4000–5000 years old (Hall-Spencer et al., 2002), are the type of marine fauna requiring the longest recovery time.

Some estimated recovery rates from bottom fishing reported in the scientific literature may be overly optimistic because of biases in the sampling design of many studies. Frequently, these studies involve trawling a small area (<50 m width) located inside a largely undisturbed site. In the ecological literature, small-scale disturbances surrounded by large, unaffected areas are often referred to as type-1 disturbances. Because of the small spatial scale of a type-1 disturbance and the low temporal frequency of such disturbances, recolonization may occur through immigration from adjacent undisturbed areas or vegetative growth (Auster and Langton, 1999; Kaiser et al., 2002). Recovery through localized immigration and vegetative growth requires less time than would be necessary if recovery were to occur through larval settlement and *in situ* reproduction of remaining organisms within the disturbed area. In the case of type-2 disturbances where the ecological community is perturbed across large areas interspersed amongst small unaffected patches, recovery usually proceeds by the slower process of larval settlement, a process where most larvae originate from either distant areas or from the small unaffected patches. This latter scenario more realistically describes the recovery process after disturbance from large-scale bottom-fishing operations on Georges Bank.

Our study is noteworthy because little research has been published on the long-term processes governing recovery from disturbance among colonial epifauna on the continental shelf of the northwest Atlantic. After the establishment of CA-II, several colonial and noncolonial taxa underwent successive increases and declines in abundance at site 17, thus, providing potential evidence of ecological succession. During 1997, bushy bryozoans briefly peaked in abundance at the shallow undisturbed site. Bushy bryozoans were able to react

quickly after establishment of a closed-area because the life span of individual colonies typically does not exceed one year (Hughes, 1989). The subsequent decline in the percent cover of this taxon may be linked to either increased interspecific competition or greater predation pressure at site 17. Organisms with life spans of five years or greater (i.e., sponges, *P. magellanicus*, *Pagurus* spp., *Asterias* spp., and *S. droebachiensis*) either increased throughout the duration of this study at site 17 or maintained a heightened level of abundance for several years before eventually experiencing a decline in numbers. Because differences between areas inside and outside CA-II, in terms of both sponge and bushy bryozoan cover, became apparent in 1997 (as shown by the error bars in Fig. 2), this finding may indicate that there is an approximately two-year lag in the initial response of colonial epifauna residing in gravel habitat to the fishing closure. This lag implies that even infrequent trawling and dredging can lead to prolonged changes in colonial epifauna composition.

Although no other research on Georges Bank has examined long-term patterns of recovery from disturbance among epifauna, our results are similar to those of two studies conducted at Cashes Ledge (located 130 km east of Gloucester, MA) on the colonization of artificial and disturbed natural substrate by colonial organisms over a period of less than two years (Sebens et al., 1988; Witman, 1998). In an area with rocky substrate at 30 m depth, the seafloor was first colonized by erect bryozoans (*Crisia eburnea* and *Idmidronea atlantica*) and the polychaete *Spirorbis* spp., while later colonizers included the ascidians *Aplidium pallidum* and *Ascidia callosa* (Witman, 1998). At the end of the 15-month duration of the Witman study, bryozoans covered approximately 35% of the disturbed patches of the seafloor, whereas no other species had yet colonized more than 6% of this area. It was predicted that it would require seven years or more for this epifaunal community to return to its natural state where sponges, sea anemones, and ascidians were the dominant fauna (Witman, 1998). In a similar study where four depth strata were examined, bushy and encrusting bryozoans were again the first organisms to settle on barren substrate; smaller patches of *F. implexa* and *Spirorbis* spp. were also early colonizers; colonization by *Aplidium* sp. and crustose coralline algae occurred later (Sebens et al., 1988). At the 50-m site on Cashes Ledge, which is the most comparable to our study area in CA-II, *I. atlantica* covered over 50% of the substrate within one year, and the cover of bryozoans approached 100% by the second year. Although bryozoan cover at our study sites never reached the extremely high percentages observed by Sebens et al. (1988), our results mirror theirs and those of Witman (1998) in that bryozoans were the predominant space holder in areas subjected to recent disturbance, and all other species of colonial epifauna never exceeded a mean cover of 5%. The recovery of the benthic community at Cashes Ledge may differ somewhat from Georges Bank, because 1) certain epifaunal species may prefer the rocky substrate at Cashes Ledge over the gravel substrate of Georges Bank (or vice versa); 2) different currents in these areas may affect the settlement rate of invertebrate larvae, and 3) the spatial scale of disturbance at Cashes Ledge (i.e., 25 cm^2) was much smaller than at Georges Bank.

Some evidence indicates that ecological succession is governing the recovery of epifauna in CA-II, but several generations of data on key benthic species would be needed to conclusively rule out other possible mechanisms controlling recovery. There are two models that describe how marine ecosystems may recover from past bottom-fishing disturbance (Auster and Langton, 1999): 1) Ecological succession may occur where the seafloor is colonized by benthic species in a predictable pattern, namely opportunistic species arrive first only to be later out-competed by climax species; 2) The lottery hypothesis, first proposed by Sale (1978), indicates that the benthic community will be dominated by species whose larvae are the first to colonize the seafloor immediately after a disturbance. This scenario implies that recovery will be greatly influenced by unpredictable, stochastic events. Because many species in CA-II have life spans of ten years or more (e.g., the Northern seastar *Asterias vulgaris*, *B. undatum*, *P. magellanicus*, *S. droebachiensis*; Hermsen et al., 2003), it is not yet possible to use time-series data to quantitatively differentiate between these two hypotheses. However, there are indications that recovery from physical disturbances is not characterized by as much stochasticity as implied by the lottery hypothesis. This lack of stochasticity is implied by the fact that bryozoans are early colonizers at both CA-II and Cashes Ledges. Another tenet of the lottery hypothesis is that early colonizers are able to consistently out-compete late colonizers by denying them access to vital habitats (Munday, 2004). This corollary of the lottery hypothesis is not met on Georges Bank, because encrusting bryozoans can be overgrown by the colonial tunicate *Didemnum* sp. and sponges (Valentine et al., 2007).

After examining six years of data collected since the establishment of CA-II, we do not know whether recovery of colonial epifauna is complete at site 17. Although large increases in the cover of colonial epifauna were not seen during 1999 and 2000, the abundance of several noncolonial species continued to grow. This continued growth of noncolonial species could in turn affect colonial epifauna composition as recovering noncolonial organisms may increasingly compete with colonial taxa for resources or prey more heavily on them. The recovery of some colonial species may still be constrained by the fact that their larvae only disperse short distances ($\leq$50 m) from parent colonies (Hughes, 1989). The final species composition of site 17 is likely to differ from that of undisturbed site 20, because the faster current speeds at the shallow site 17 can transport sand across the gravel pavement, inhibiting the growth of arborescent epifauna and *F. implexa*. In fact, the scouring caused by sand transported to site 17 during a winter storm that coincided with our

November 2000 research cruise may be responsible for the low densities of certain species (e.g., sponges) observed during that year. Overall, an extended period of monitoring is needed to determine which epifaunal species will be most abundant in CA-II once recovery is complete.

In conclusion, the cover of hydroids, bushy bryozoans, sponges, and *F. implexa* was generally higher at sites undisturbed by mobile fishing gear, although the magnitude and significance of this effect depended on water depth and differed between years. Among colonial epifauna taxa, only encrusting bryozoa are positively affected by bottom-fishing disturbance. The species of colonial epifauna and noncolonial megafauna that are more abundant at undisturbed sites generally possess characteristics that make them vulnerable to the effects of mobile fishing gear. These characteristics include emergent growth forms, soft body parts, low motility, use of structurally complex microhabitats, long life spans, slow growth, and larval dispersion over short distances. Following the prohibition on bottom fishing in CA-II, both colonial (i.e., sponges and bushy bryozoans) and noncolonial (i.e., *P. magellanicus*, *S. droebachiensis*, *Pagurus* spp., *Asterias* spp.) organisms increased in abundance. Although research investigating the long-term recovery of epifauna from bottom-fishing disturbance is ongoing in the other areas of the Gulf of Maine (e.g., Stellwagen Bank and western Gulf of Maine closed area), this study is one of the first in the region to track the recovery of the colonial epifaunal community from disturbance for a period extending beyond two years. The fact that it took most taxa two years or more to initially respond to the cessation of bottom fishing indicates that even infrequent trawling can alter benthic communities for years to come. When monitoring of CA-II was first initiated, it was predicted that it would take 5–10 years for the megafaunal community to recover (Collie et al., 1997). After six years of studying transects inside CA-II, we conclude that recovery does not yet appear complete and that the higher end of this projection may prove more accurate.

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Pelagic behavior of adult Greenland halibut (*Reinhardtius hippoglossoides*)

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Abstract—It is evident from several field experiments with vertical longlines and archival tags, as well as concurrent studies of predator-prey relationships, that adult specimens of the deep-water flatfish Greenland halibut (*Reinhardtius hippoglossoides*) make regular excursions several hundred meters through the water column. The distribution of longline catches within the water column is confined to a well-defined depth layer overlapping with the distribution of blue whiting (*Micromesistius poutassou*), an important prey species, and depth recordings from archival tags overlap with Atlantic herring (*Clupea harengus*), the other major fish prey. The degree of pelagic use varies with fish size as well as seasons. Smaller individuals are found further off the bottom, and pelagic activity is greatest during early autumn. Interaction with pelagic prey species can influence results from bottom trawl surveys.

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Greenland halibut (*Reinhardtius hippoglossoides* (Walbaum)) is a deep-water flatfish of high commercial value in the northernmost parts of the Atlantic and Pacific oceans. The absence of a swim bladder renders the species virtually invisible to echo sounders; therefore data from scientific and commercial fishing operations conducted close to the seabed with trawls, long-lines, and gill nets are used for stock assessments (Bowering and Nedreaas, 2000). This practice may be surprising, given the general perception of Greenland halibut as a more free-swimming species than other flatfishes (Merrett and Haedrich, 1997). If indeed the use of the pelagic zone by this species is extensive or frequent, results from bottom trawl surveys may be biased and less informative for use in assessments. This problem increases if the pelagic behavior varies over time or is unevenly distributed among sizes, the sexes, and maturity groups.

The perception that Greenland halibut may be a pelagically distributed species has primarily been based on assumptions, anecdotical information, and circumstantial evidence. Morphological characters such as a streamlined body shape, pigmentation of the blind side, and positioning of the left eye on the dorsal ridge of the head led de Groot (1970) to suggest that Greenland halibut behaves more like a roundfish. This has been supported by historical reports of specimens of Greenland halibut being caught in pelagic waters with hand lines and salmon drift nets (Christensen and Lear, 1977; Merret and Haedrich, 1997). More recent documentation of pelagic use is scarce, particularly for adult specimens.

Jørgensen (1997) reported that one-year-old Greenland halibut were commonly found in pelagic trawls in West Greenland waters, and two-year-olds appeared much less frequently. Older individuals were virtually absent from pelagic trawls, even when they were captured in high abundance in bottom trawls. The author concluded, however, that these results may have been influenced by differences in the selectivity patterns of bottom and pelagic trawls.

Although records are scarce, a significant portion of the diet of adult Greenland halibut consists of bathy- and mesopelagic fish, mollusks, and crustaceans such as redfish (*Sebastes* spp.), blue whiting (*Micromesistius poutassou*), cephalopods, shrimps, and euphausiids, as well as epipelagic species such as Atlantic herring (*Clupea harengus*) and capelin (*Mallotus villosus*) (Bowering and Lilly, 1992; Dawe et al., 1998; Michalsen and Nedreaas, 1998; Hovde et al., 2002; Solmundsson, 2007). We therefore used the assumed feeding modus of pelagic

Greenland halibut to study their pelagic distribution. A series of experiments with vertical longlines were designed to catch the species in the pelagic zone, and information on individual depth trajectories were obtained from archival tags for comparisons with the vertical distribution of longline catches. In addition, stomach contents from concurrent bottom trawl hauls provided information on diet of Greenland halibut, length compositions were obtained from demersal longlines and bottom trawl, and acoustic surveys gave insight into the pelagic distribution of some of the prey species. The study aims at describing the use of the pelagic zone by adult Greenland halibut in the Northeast Arctic, both in terms of temporal variability and through parameters such as size, sex, and maturity.

Materials and methods

Data were mainly collected from dedicated surveys with hired commercial longline vessels within a limited area of the continental slope between northern Norway and Bear Island (the central study area, Fig. 1). The longline vessels were used for pelagic fishing with vertical longlines, fishing with demersal longlines, and release of fish tagged with archival tags. Data were also collected from a number of additional surveys in the same or nearby areas and time periods. These additional surveys included bottom-trawl surveys for stomach contents analyses and length-frequency distributions, and trawl acoustic surveys for acoustic profiles. The additional surveys were not originally designed for this study, and data from these investigations were extracted for the extended survey area (Fig. 1).

Vertical longlines

Vertical longline experiments were conducted within the central study area during seven surveys in March, August, and November from 2003 through 2005 (Fig. 1). The main survey, with 73% of all the vertical longline settings, took place in August 2005. During the first six surveys the experiments were only a minor part of the total mission. These six were made partly to gather experience, and partly to establish a time series to be compared with the main survey.

The vertical longline gear consisted of an anchor (25 kg minimum), a rope, a hooked longline, another rope and, finally, a buoy at the surface. A Mustad autoline system was used on all vessels. The hooked longline was 9 mm thick and had hooks of type Mustad EZ 12.0, ganglion length was approximately 50 cm, and the spacing between hooks was 1.5 meters. To record the position and action of the vertical longline, archival tags (DST-milli, Star Oddi, Reykjavik, Iceland) were attached to the top and bottom of the hooked section, as well as over the anchor. Bait was a 1:1 ration of Atlantic mackerel (*Scomber scombrus*) and flying squid (*Todarodes sagittatus*)—the most common bait types used to target catch of Greenland halibut (Bjordal and Løkkeborg, 1996). Bait size was approximately 2.5 cm × 5 cm.

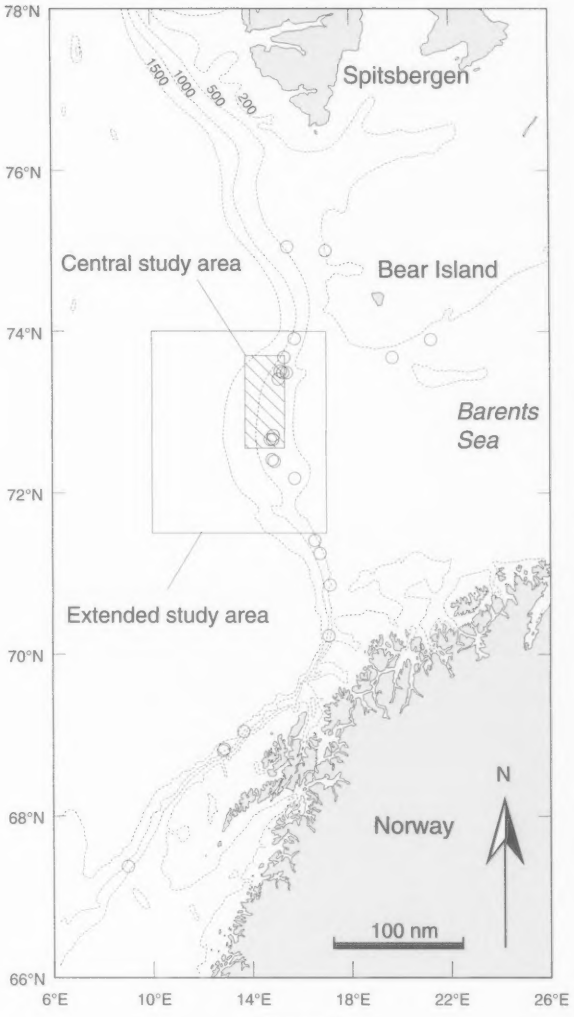

Figure 1

Bathymetric map of both the central and expanded study area situated along the continental slope between Norway and Spitsbergen. Depths are in meters. The longline experiments, as well as release of Greenland halibut (*Reinhardtius hippoglossoides*) tagged with archival tags, were made within the central study area, whereas data from the acoustic surveys and bottom trawl surveys were gathered from the extended study area. Recapture positions of individuals with archival tags are marked with open circles.

The hooked section of the longline covered most of the water column, from 45 m below the surface to 20–150 m over the bottom. Longlines were rigged to ensure that the hooks did not touch the bottom, and the position of the anchor and the highest and lowest hooks were monitored by using archival tags. At bottom depths

greater than 1000 m, a drifting vertical longline with no anchor was used. Soak time ranged from 3 to 33 hours. During the main survey in August 2005 mean soak time was 12.6 (±6.1 standard deviation [SD]) hours.

The vertical longlines were rigged to optimize the vertical stability. During the main survey in August 2005 the anchor settled on the seafloor within 10–15 minutes, and the vertical longline settled at the proper depth after about 30 minutes. Once settled, depth of the lowest hook typically had a 2SD of 15–45 m. Stability increased for hooks higher up in the water column (Fig. 2). With the exception of the few deepest hooks, which at some settings may have been close to the seabed for a few minutes, hooks were at a pelagic depth throughout the soak time. During earlier cruises the vertical stability was more variable, with 2SD ranging from 2 to 150 m. The hauling process was completed in approximately 30 minutes in all experimental periods.

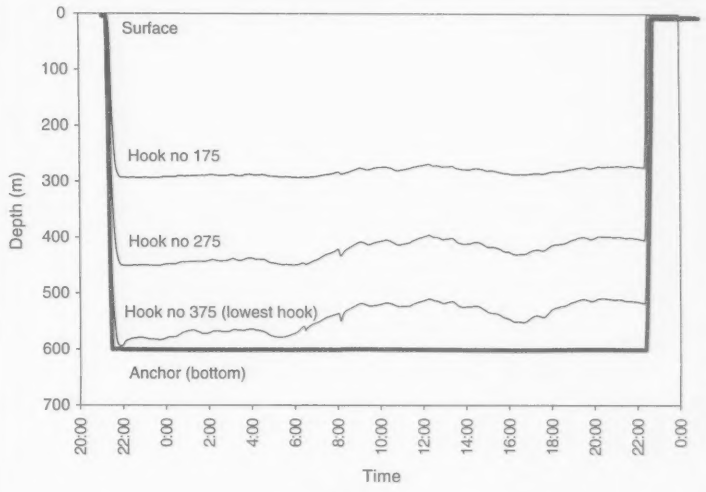

Figure 2

Depth profile of a vertical longline from August 2005. One archival tag was attached to the anchor and another three to the vertical longline at different intervals. Depths were recorded every 3 minutes from time of setting to hauling. Vertical movements of this longline were larger than those for most longlines from August 2005 (2 standard deviations of mean depth for the lowest hook=51 m, versus 15–45 m for most August 2005 longlines).

All fish caught on vertical longlines were classified to species, and the catch depth D_c was calculated as follows:

$$D_c = D_h + (D_l - D_h)H_c/H_{max},$$

where D_h and D_l = the mean depths of the highest and lowest hook as measured by archival tags;
H_c = the hook number of the catch; and
H_{max} = the total number of hooks on the hooked section.

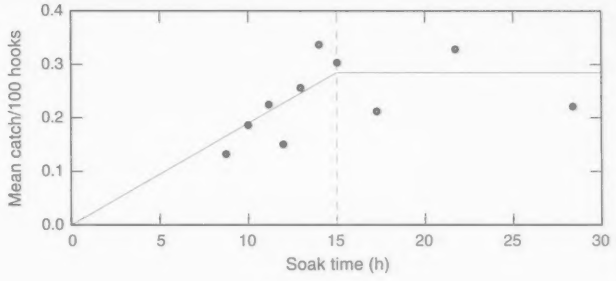

Figure 3

Observed relationship between mean catch (in number) of Greenland halibut (*Reinhardtius hippoglossoides*) and soak time (1-hour groups) for vertical longlines. n=221. The solid line represents the saturation model used. All means were based on groups of nine or more vertical longlines from August 2005.

For all Greenland halibut a standard biological sampling was performed (length, weight, sex, maturity, and stomach fullness). Stomachs with contents were frozen and analyzed in the laboratory. Prey items were counted, weighed, and determined to the lowest possible taxonomic level. The digestion status was noted according to a five-point scale: 1) fresh, 2) digestion just started, 3) moderately digested, 4) highly digested, and 5) digestion almost complete. Longline bait was easily recognized and excluded from the analysis.

According to data from August 2005, the catch rate of vertical longlines increased with soak time until saturation was reached after approximately 15 hours (Fig. 3). Fishing effort, E_{ijk}, generated by a single vertical longline k during setting time T_k within a given combination of fishing depth interval i and bottom depth interval j was thus defined as

$$E_{ijk} = T'_k \times H_{ijk}/100,$$

where $T'_k = T_k$ if $T_k < 15$ hours;
T'_k = 15 hours if $T_k \geq 15$ hours; and
H_{ijk}= the number of hooks.

Table 1

Overview of recaptured (Rec.) archival tags from Greenland halibut (*Reinhardtius hippoglossoides*). Fish total length (TL, cm) measured during tagging. F=female; M=male.

Rec. no.	Interval between recording (min)	Release date	No. of days recording	Fish TL	Fish sex
1	60	30.11.02	687	67	—
2	60	08.12.02	334	64	F
3	60	08.12.02	24	68	—
4	60	08.12.02	188	61	—
5	60	08.12.02	12	54	M
6	60	12.12.02	18	67	F
7	60	13.12.02	16	67	F
8	60	13.12.02	70	74	F
9	10	12.08.03	151	57	—
10	10	14.08.03	149	57	—
11	15	14.08.05	306	76	F
12	15	14.08.05	120	63	F
13	15	15.08.05	54	66	F
14	15	15.08.05	67	77	F
15	15	15.08.05	296	74	F
16	15	15.08.05	10	57	—
17	15	15.08.05	8	73	F
18	15	15.08.05	51	55	F
19	15	16.08.05	54	63	F
20	15	16.08.05	61	75	F
21	15	16.08.05	177	77	—
22	15	16.05.06	261	67	—
23	15	16.05.06	261	47	F
24	15	16.05.06	99	68	F
25	15	17.05.06	196	65	—

Total effort within ij was the sum of E_{ijk} for k=(1 to N_{ij}), where N_{ij} is the number of vertical longlines in ij.

Demersal longlines

Length-frequency distribution data of Greenland halibut on the bottom (separated by sex) were collected by using demersal longlines. In August 2005 the demersal longlines were 3.5 km long and were deployed in an east-west direction perpendicular to the continental slope. Fishing depth ranged from 450 to 950 m (mean of 655 [±155] m). Soak time ranged from 3 to 22 hours (mean of 10.7 [±5.2] hours). The hooked section on demersal longlines was identical to that of vertical longlines.

Archival tags

On four occasions (November 2002, August 2003, August 2005, and May 2006) a total of 503 Greenland halibut were tagged with archival tags (DST milli or DST Pitch and Roll, Star-Oddi, Reykjavik, Iceland). All individuals were tagged from demersal longlines within the central study area. The longlines were carefully hauled and only individual fish in apparently good shape were tagged and then immediately released. Recaptures were made with bottom trawls, mainly along the continental slope (Fig. 1). The tags were programmed to record time and ambient depth (pressure) every 10, 15, or 60 minutes, and the depth trajectories were recovered after recapture. Up to October 2007, a total of 38 (7.6%) of the released tags were recaptured. Of the recaptured tags, 13 had been destroyed and there was no possibility of data extraction. Of the 25 tags containing data, three tags had stopped recording after being exposed to depths greater than 1000 m, and six tags had stopped recording because the memory was full. For the remaining 16 tags, data were recovered for the whole period from release to recapture. Table 1 shows recording sequence, number of data sampled, as well as sex and length of the tagged individual for all 25 tags used in the analysis. These tagging experiments will form the basis for more detailed future publications; here we restrict ourselves to consider the vertical activity and compare it with the results from the vertical longline experiments.

The tags, by design, conveyed no direct information on bottom depth or the distance from the bottom. However, it was assumed that readings of constant ambient depth can only occur when the fish are on the bottom,

whereas data showing frequent changes in vertical position may indicate pelagic swimming behavior. The vertical activity was analyzed in two ways: standardized vertical distance and vertical swimming activity. Standardized vertical distance (*VD*) between succeeding observations of ambient depths (*D*) were standardized to equal time intervals:

$$VD_i = 3600 \times \text{abs}(D_i - D_{i-1})/s,$$

where s = the time step (in seconds) between observation i–1 and i.

For each individual fish and day, the extent of vertical swimming activity (*VA*) was estimated as

$$VA = \Sigma(D_{max,i} - D_{min,i})/12,$$

Where $D_{max,i}$ and $D_{min,i}$ represents the maximum and minimum depths recorded within a two-hour time interval i, and the summation is across the twelve two-hour time intervals within each day.

Thus *VA* is not only dependent on the vertical range traveled during a day, but also on the frequency of vertical excursions throughout the day. Whereas *VD* measures the speed during a depth change, *VA* is a measure of activity through the day.

Additional surveys

Trawl acoustic surveys were conducted in the Barents Sea and Norwegian Sea in August 2003–2005, as part of a regular monitoring program of pelagic fish stocks in these areas. In the present study, data from these investigations were extracted for the extended survey area. The surveys were conducted with a Simrad EK 60 echosounder, frequency 38 kHz (Kongsberg Maritime AS, Horten, Norway). The acoustic recordings were scrutinized each day by using the Bergen Echo Integrator, and allocation of S_A (nautical area scattering coefficient) values on species was made by trained personnel using standard procedures (Korneliussen, 2004).

Bathymetric distributions of selected species of fish in bottom trawls were used for comparisons with the pelagic records. Data were gathered from the annual Norwegian Greenland halibut surveys in August 2003–2005, within the extended study area. In these surveys an Alfredo-5 commercial bottom trawl with rockhopper ground gear (Engås and Godø, 1989) and a measured mean vertical opening of 3.8–4 m was used at towing speeds of 3.5–4.0 knots. Mesh size in the codend was 60 mm.

Stomach-contents data were collected from the same surveys, as well as on similar surveys in November 2003 and March 2004. A standard biological sampling (length, weight, sex, maturity, and stomach fullness) was performed for two individuals in every 5-cm length group. Individuals sampled were chosen randomly, except in August 2005 when only individuals with stomachs containing food were sampled. These stomachs were frozen and analyzed in the laboratory. Methods were the same as those used for stomachs sampled from vertical longline catches.

Results

Pelagic catches of Greenland halibut

Greenland halibut were caught on vertical longlines in most parts of the water column, but despite high effort, the species was rarely recorded in the upper few hundred meters of the water column. Throughout the experimental period a total of 283 individuals were caught, and the species was recorded during all surveys but one. Catch depth ranged from 208 to 934 m (Fig. 4). Seventy percent of all catches were from the main survey in August 2005, when 93% of the individuals were caught between 400 m and 800 m depth. There seemed to be an upper catch limit which was relatively stable across bottom depths. In August 2005 this limit was 300 m at 600–700 m bottom depth, 400 m at 800–1000 m bottom depth, and 500 m at 1200 m bottom depth. The westward distribution boundary could not be identified because of inadequate coverage of large depths.

Greenland halibut was caught at shallower depths in November than in either March or August. For March and August surveys, mean catch depth above 400–700 m bottom depth ranged from 484 m to 554 m. For the two November surveys, the corresponding numbers were 378 m and 363 m, respectively, differing significantly from all individual March and August surveys (*t*-tests, $P<0.001$). The two November surveys were also the only surveys where fish were captured in the region above 300 m catch depth (Fig. 4).

There were no clear or significant changes in length composition with catch depth, but rather with bottom depth and with distance from the bottom (data from August 2005, Fig. 5). This was partly due to males, which are smaller than females, being distributed higher up in the water column (*t*-tests, $P<0.001$) and partly to a significant decrease of female length (linear regression, $P<0.05$). Below 100 m from the bottom, approximately 50% of the catches were males, whereas above 300 m, 75% were males.

The length range of individuals caught on vertical longlines was broad (41–85 cm), but the length and sex composition seemed to have more in common with bottom trawl catches than bottom longline catches (Fig.6). Bottom longlines caught few fish smaller than 50 cm, which were primarily males. Bottom trawls, on the other hand, caught fish as small as 30 cm length, indicating that both sexes were available at the bottom in approximately equal shares.

During individual surveys in March and August, the proportion of females in the pelagic longline catches varied from 8% to 48% (mean of 35%). In November no females were caught—a finding that differed significantly from all March and August surveys (χ^2, df=1,

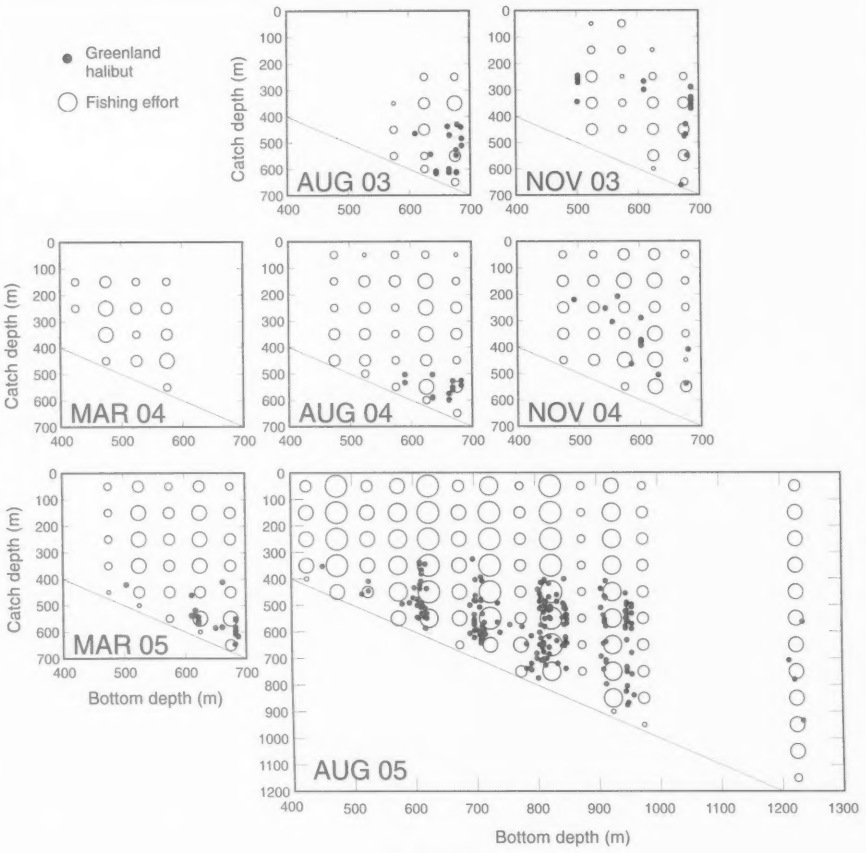

Figure 4

Pelagic catches (noted as individuals) of Greenland halibut (*Reinhardtius hippoglossoides*) on vertical longlines. Individual catches are shown by bottom depth (m) and catch depth (m). Fishing effort is shown for each 100-m catch depth interval and 50-m bottom depth interval. The area of the fishing effort symbol is proportional to fishing effort (hooks × hours/100). The straight diagonal line represents the bottom.

$P<0.01$). In November all Greenland halibut caught were classified as late maturing or ripe, whereas in catches from March and August all but three individuals were immature or early maturing.

Individual depth trajectories

Of the 25 recaptured fish with intact archival tags, the time recorded varied from a few weeks up to two years, yielding a total of 296,000 depth recordings. All tags were both released and recaptured at or close to the continental slope (Fig. 1). For all recordings combined, the 25th, 50th, and 75th percentiles of depth were 454, 522, and 617 m, respectively. Six percent of all recordings were from depths above 300 m, from 11 recaptures. For individual tags this percentage ranged from zero to 20%, with a mean of 2.8%. One individual was regularly recorded above 100 m depth, and seven individuals were recorded below 1000 m. Only one of the 16 individuals, for which sex was determined, was male.

Most of the recaptured tags revealed alternating periods of distinctly different vertical activity by Greenland halibut. Such periods were apparent on both small (hours-days) and large (days-months) scales. Diurnal variation was most apparent at depths above 300 m (Fig. 7). Here the occurrence of large standardized vertical distance (VD) between successive records was three times higher at night (21h–03h) than during the midday (09h–15h), and records of vertical inactivity ($VD<0.5$ m per hour) were twice as numerous during daytime (Fig. 7). Above 300 m, the total number of observations was 19% higher at night

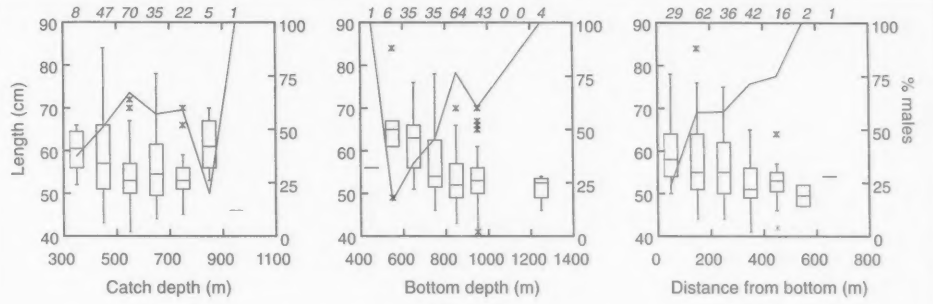

Figure 5

Line plot of sex composition and box plot of length of Greenland halibut (*Reinhardtius hippoglossoides*) caught on vertical longlines, by catch depth, bottom depth, and catch depth in distance from the bottom (all in 100-m intervals). Data are from August 2005. The number of individuals is given above plots. The plots show median, inter quartile range, upper and lower fence, and outliers as defined in SYSTAT 11 (SYSTAT Software Inc., San Jose, CA). Note that % males and % females add up to 100%.

time than during the day, indicating that many of the Greenland halibut in the upper 300 m during night may migrate to deeper waters during the day. The higher number of observations early in the day in the 300–500 m depth zone, and later in the day at depths below 500 m support this (Fig. 7). The vertical activity increased and the diurnal signal weakened with increasing depth. The number of recaptures was not considered high enough to warrant analyses of individual and seasonal differences in the diurnal activity pattern.

The larger-scale time-variability is illustrated in Figure 8, which shows the depth trajectory of five tagged fish, together representing the typical pattern seen in all tags. In most cases periods of high vertical activity began with a sudden descent to much deeper depths than those occupied at any time during the periods of low vertical activity. This pattern is clearly apparent from examples A1, A2, and C1 in Figure 8, where periods of low vertical activity at depth less than 500 m suddenly changed to periods of high-frequency changes in depth between 500 and 800 m. Example A3 shows a trajectory from a tag that stopped recording after a sudden descent to depths greater than 1000 m, which is the depth limit of the tag. This cessation in recording happened to three of the 25 recovered time series.

The degree of high vertical swimming activity (*VA*) varied throughout the year (Fig. 9). The proportion of days with high vertical activity was greatest during August–October and least in January and February.

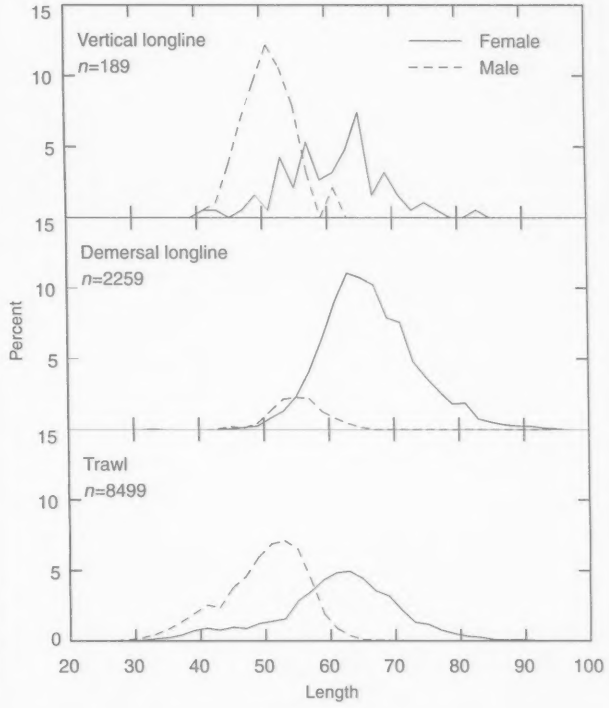

Figure 6

Length-frequency distribution (2-cm length groups) of female and male Greenland halibut (*Reinhardtius hippoglossoides*) caught by vertical longlines, conventional demersal longlines, and demersal trawl. Data are from August 2005. Bottom depths covered were 451–1235 m for vertical longlines, 349–932 m for demersal longlines, and 433–1325 m for trawl.

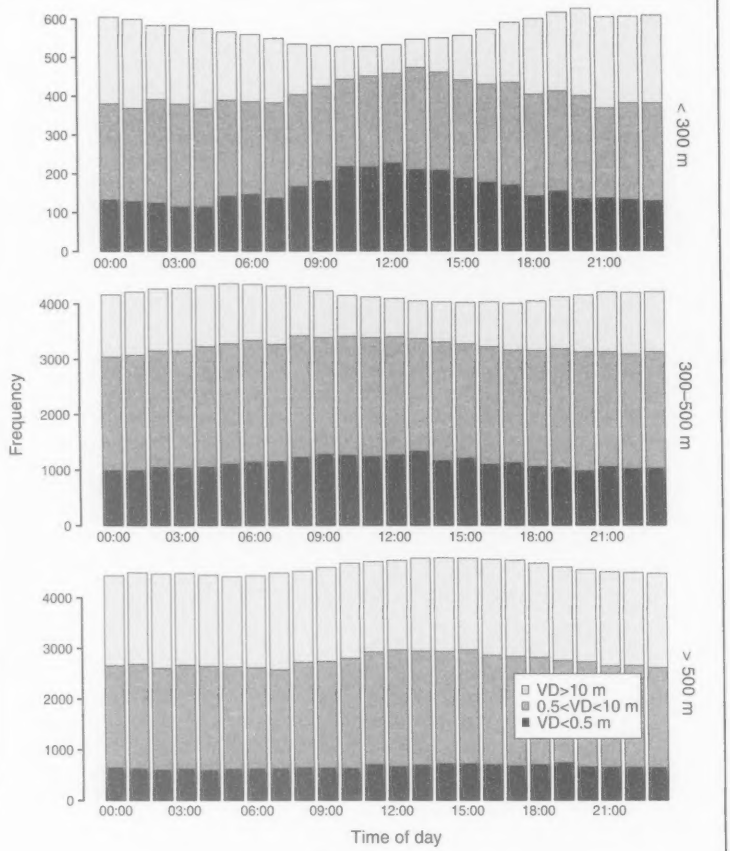

Figure 7

Diurnal variations in standardized vertical distance (*VD*, in meters) traveled by Greenland halibut (*Reinhardtius hippoglossoides*) tagged with archival tags, within each of three depth intervals. The different shaded bars represent frequencies of high (>10 m), medium (0.5–10 m), and low (<0.5 m) *VD*, respectively.

Prey composition

The main prey categories found in Greenland halibut stomachs were crustaceans, cephalopods, and fish. These species all occurred in 35–47% of stomachs with contents (all trawl surveys combined, Table 2). Crustaceans were mainly shrimps (primarily *Pasiphaea* spp. and *Pandalus borealis*) and gammarid amphipods, whereas *Gonatus fabricii* was the predominant cephalopod. Herring and blue whiting were the most common fish prey. One stomach contained a fish tag that one month earlier had been attached to a 19-cm salmon (*Salmo salar*), but whether this tag represents a direct or indirect foraging link between salmon and Greenland halibut, remains unknown (Rikardsen et al., 2008).

Of the three main categories of prey, Crustacea were a relatively stable contributor, whereas frequency of occurrence of cephalopods and fish varied between surveys (χ^2, df=4, P<0.01). This temporal variability was mainly due to three species or species groups. *Gonatus fabricii* was recorded more frequently in August than in March and November, whereas herring was most frequent in August 2003 and March 2004, and blue whiting in August 2003 and 2004.

Figure 10 shows how the diet changed with the size of the predator. In terms of weight, the contribution of cephalopods, shrimps, and most other crustaceans decreased with increasing length of Greenland halibut. On the other hand, the contribution of herring and most other fish increased with increasing predator length. Eelpouts and flatfish were found only in Greenland halibut specimens larger than 65 cm, and redfish were eaten only by individuals larger than 75 cm.

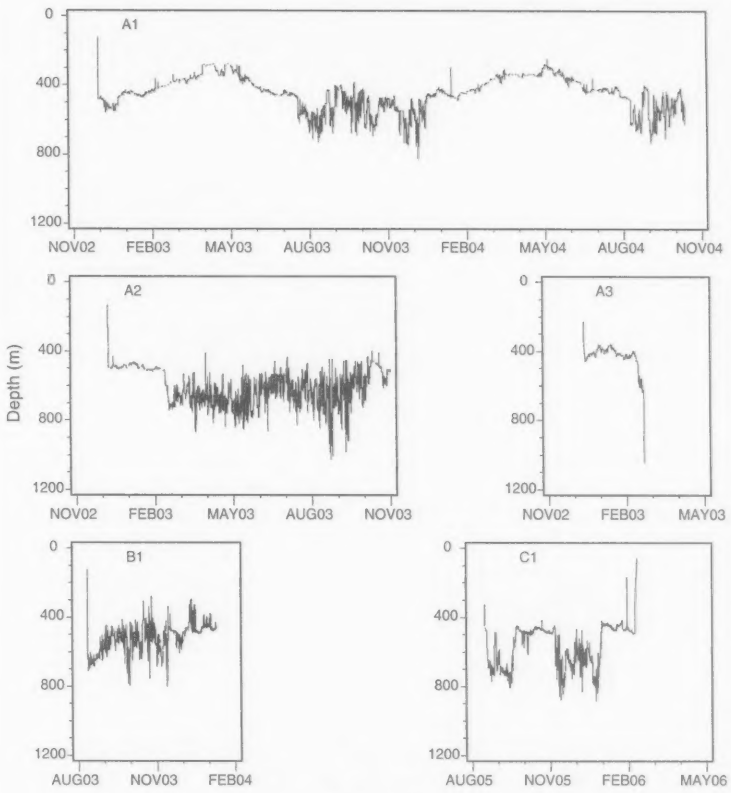

Figure 8

Examples of individual depth trajectories determined from archival tags on recaptured Greenland halibut (*Reinhardtius hippoglossoides*). Examples A1–A3 from release in November 2002, example B1 from release in August 2003, and example C1 from release in August 2005. For A3 the recordings stopped before recapture, probably because the fish descended beyond the depth limit that the tag was designed to withstand.

Of all Greenland halibut caught on vertical longlines in August 2005, only 27 individuals, or 14%, had recently been feeding, as evidenced by the presence of prey in the stomachs. This percentage ranged from 14% to 40% for fish caught in bottom trawls (Table 2). Stomachs from specimens caught on vertical longlines contained food from all major prey groups that were also found in stomachs of trawl-caught fish. Of these specimens, 33% contained fresh prey, i.e. prey without signs of digestion. For trawl-caught fish during the same period this percentage was significantly lower; only 7% contained fresh prey (χ^2, df=1, $P<0.01$). In terms of percentage by number, 17% of the prey items from vertical longlines and 5% from bottom trawl catches were classified as fresh (χ^2, df=1, $P<0.01$). The fresh prey of Greenland halibut caught by vertical longlines were *G. fabricii* and the hyperiid amphipod *Parathemisto abyssorum*. The Greenland halibut were all captured in waters above an 800–900 m bottom depth, and 70% of them were caught more than 300 m above the bottom. The fresh contents from trawl catches consisted of hyperiid amphipods, *G. fabricii*, and *Pasiphaea* sp., as well as one case of offal. These individuals were caught at 600–1200 m depth.

Pelagic distribution of potential prey

The acoustic profiles from the extended study area in August 2003–05 were all quite similar, with an acoustic layer in the upper 50 m and another around 300 m depth (Fig. 11). Data from 2005 showed that this pattern continued out to the deeper part of the slope. That year the echo sounder was regrettably set to record only down to 500 m depths, but data from 2003 and 2004 strongly indicated that very little backscatter should be expected below 500–600 m.

Table 2

Percent frequency of occurrence of prey categories determined from stomach contents of Greenland halibut (*Reinhardtius hippoglossoides*) caught on five individual bottom trawl surveys in August 2003–05, November 2003, and March 2004, and on the vertical longline survey in August 2005, and for all bottom trawls combined.

	Aug 2003 bottom trawl	Nov 2003 bottom trawl	Mar 2004 bottom trawl	Aug 2004 bottom trawl	Aug 2005 bottom trawl	Aug 2005 vertical longline	Combined bottom trawl
Crustaceans							
Amphipods	4.5	5.4	6.7	7.1	14.7	22.2	7.8
Euphausiids	0.0	5.4	0.7	2.4	0.0	0.0	1.1
Shrimps	28.8	35.1	15.3	21.4	36.0	48.1	24.6
Undetermined	3.0	8.1	24.7	7.1	13.3	3.7	14.9
Cephalopods							
Gonatus fabricii	37.9	10.8	20.0	35.7	58.7	40.7	34.6
Rossia spp.	0.0	0.0	1.3	2.4	1.3	3.7	1.1
Undetermined	1.5	0.0	4.7	2.4	4.0	0.0	3.5
Fish							
Salmon [1]	0.0	0.0	0.0	0.0	1.3	0.0	0.3
Herring	18.2	8.1	36.0	2.4	5.3	0.0	20.0
Blue whiting	9.1	5.4	2.7	14.3	2.7	0.0	5.4
Sebastes spp.	3.0	0.0	0.7	0.0	0.0	0.0	0.8
Other fish	0.0	5.4	1.3	4.8	2.7	0.0	2.2
Undetermined	21.2	32.4	31.3	14.3	12.0	3.7	23.8
Offal	3.0	0.0	0.0	4.8	4.0	0.0	1.9
Other	3.0	2.7	1.3	11.9	1.3	0.0	1.8
Number of stomachs examined	327	98	378	298	—	—	198
Stomachs with contents	66	37	150	42	75	370	27
Empty stomachs	80%	62%	60%	86%	—	—	86%

[1] A Carlin dangler tag from a tagged salmon was found in one Greenland halibut stomach.

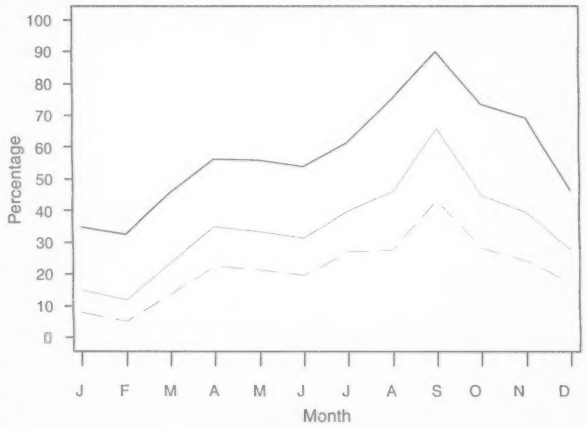

Figure 9

Seasonal variation in mean vertical activity (*VA*) for Greenland halibut (*Reinhardtius hippoglossoides*) tagged with archival tags. For each month, the number of days of individual archival tag recordings where *VA* was greater than 5 (bold), 10 (regular), and 15 m (dotted line) respectively, is given as a percentage of the total number of days recorded by all tags combined.

During these acoustic surveys, most of the total backscatter was allocated to herring in the upper layer and blue whiting in the lower layer. As usual with these types of surveys, the allocation was based on visual recognition of typical patterns, guided by data on target strength of individual echoes and catch composition from sporadic pelagic trawl hauls (mostly outside our extended survey area). Figure 11 shows how catches of redfish and gadoids on vertical longlines compare with the results from the acoustic surveys. The gadoids Atlantic cod (*Gadus morhua*), saithe (*Pollachius virens*), and blue whiting were generally caught within the depth range of the lower acoustic layer, i.e. from 200 to 500 m. The low-target-strength species, the deepwater redfish (*Sebastes mentella*), was caught in the lower part of this layer and below, mostly from 400 to 600 m depth. The bathymetric distribution of Greenland halibut catches from pelagic longlines overlapped to a large extent with the distribution of the prey species redfish and blue whiting, much less with other gadoids, and not with herring.

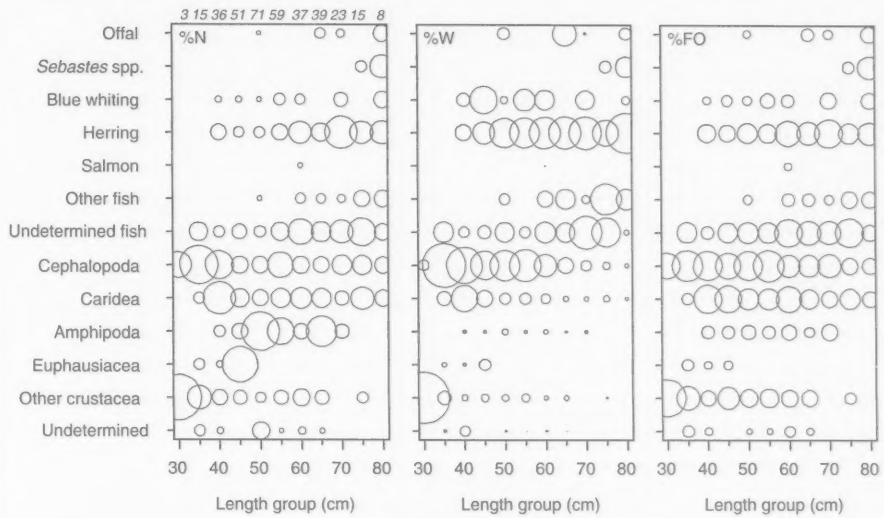

Figure 10

Prey composition by 5-cm length groups of Greenland halibut (*Reinhardtius hippoglossoides*) from bottom trawl, all periods combined. Bubble size is proportional to percentage composition by numbers (%N), weight (%W), and frequency of occurrence (%FO). Number of stomachs with contents is given above left panel.

The abundance of Greenland halibut in bottom trawl was highest between 500 and 800 m, and the species was virtually absent below 1000 m. Figure 11 also shows that the backscatter from the 10-m high bottom-zone almost disappeared below 500 m depth, when Greenland halibut, which does not have a swim-bladder and thus a very low target-strength, dominated almost completely over all other fish species. Above 500 m, blue whiting and several demersal species were numerous, whereas Greenland halibut catches were near zero.

Discussion

Greenland halibut caught in pelagic waters ranged in length from 41 to 84 cm, which is within the same range as that found for those caught on the bottom. This is in contrast to a study by Jørgensen (1997), who found that although one- and two-year-old Greenland halibut from West Greenland nursery areas were found in high abundance in pelagic waters, larger fish (>52 cm) were not found in the pelagic zone of the area inhabited by adult fish. However, Jørgensen commented that the pelagic occurrence of larger specimens may have been underestimated if these fish were able to avoid the pelagic trawl. Support for the ability of large individuals to avoid bottom trawls has subsequently been noted (Albert et al., 2003).

The influence of gear selectivity processes was apparent in length-frequency distributions of catches from demersal longlines and bottom trawls—distributions that closely resembled those found by Huse et al. (1999). The results from trawl catches indicated that small individuals were present on the bottom even though these fish were not caught by demersal longlines. The reason for this could be that the commercial longlines used in the experiment targeted large individuals by factors such as hook- and bait-size, which are important in the size-selectivity of longlines (Bjordal and Løkkeborg, 1996). Also, differences in swimming speed and in the competitive ability between small and large fish may impart a selection bias, bringing a relatively high proportion of large individuals into contact with the gear (Bjordal and Løkkeborg, 1996). Therefore, because small fish were common in pelagic longline catches, this may imply that these fish were more abundant than catch rates indicated.

The depth recordings from archival tags supported the findings from vertical longline results. The recordings showed periods of distinctly different vertical activity, both on a small scale (diurnal) and large scale (typically several weeks). Although the depth-recordings bore no information about the distance from the sea floor, it seems reasonable to assume that the high vertical activity periods were associated, at least partly, with pelagic distribution. The diurnal signal was most apparent at shallower depths, but could also be interpreted as an increase in migration to lower depths during daytime. Depth recordings from the

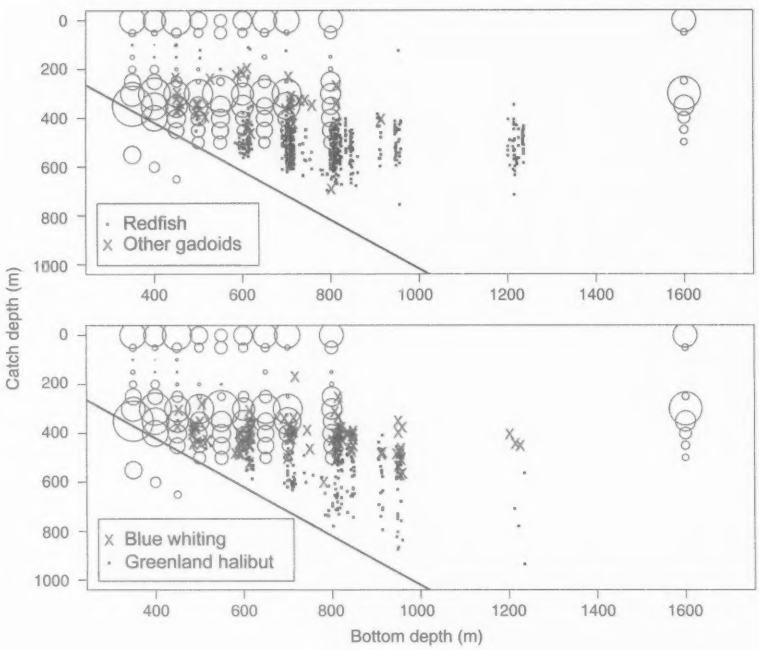

Figure 11

Total acoustic backscatter (bubbles) and catch (noted as individuals) on vertical longlines. **Upper panel** shows total backscatter together with longline catches of redfish (*Sebastes mentella*) (■) and gadoides (X); **lower panel** shows total backscatter and longline catches of blue whiting (*Micromesistius poutassou*) (X) and Greenland halibut (*Reinhardtius hippoglossoides*) (■)). Data from August 2005. Diagonal line represents the bottom, and bubbles beneath this line are acoustic recordings from the bottom channel, i.e., from the water column from the bottom up to 10 m off the bottom.

high vertical activity periods were comparable to depth distribution of vertical longline catches.

Individual and seasonal differences in diurnal activity patterns could not be investigated further due to the low number of recaptures, and due to the unbalanced sex distribution of recaptured fish. However, diurnal variability in bottom trawl catches associated with small individuals migrating into the water column at night time has already been reported off the coast of Labrador during summer (Bowering and Parsons, 1986). Such behavior is probably related to foraging, and should be expected to vary according to prey availability. This may explain why diurnal variability was not previously observed in bottom trawl catches of Greenland halibut from West Greenland waters (Jørgensen, 1997).

The predominant prey species or prey groups in stomachs of Greenland halibut captured with bottom trawl were herring, blue whiting, shrimps, amphipods, and the cephalopod *G. fabricii*. This finding is in agreement with that of previous studies from the Barents Sea-Norwegian Sea continental slope (Michalsen and Nedreaas, 1998; Bjelland et al., 2000; Hovde et al., 2002). The acoustic surveys demonstrated that the vertical distribution of blue whiting overlapped with the distribution of Greenland halibut as determined from vertical longlines. For herring, which were the most important fish prey, no overlapping distributions were evident. Herring were not recorded below 200 m depth during the trawl acoustic surveys—a depth that is shallower than any recording of Greenland halibut from vertical longlines, but archival tag recordings showed that Greenland halibut may be found higher up in the water column, up to above 100 m depth.

A broad overlap could be seen between the vertical distributions of redfish and Greenland halibut. Despite this overlap, redfish occurred only in stomachs of fish larger than 75 cm. This finding may be due to the size selectivity of prey as seen in West Greenland waters, where Greenland halibut up to 64 cm total length fed only on redfish smaller than 15 cm, even though larger specimens were present (Pedersen and Riget, 1993). In our area, redfish smaller than 15 cm have been virtually absent because recruitment has been very low since 1990.

The cephalopod *G. fabricii* and the shrimps *Pasiphaea* spp. and *P. borealis*, all important prey species, may also have been encountered in the pelagic zone. *Gonatus fabricii* juveniles are distributed in the surface layers, living gradually deeper as they become older. As adults, *G. fabricii* are largely benthic at depths of 200–3000 m, performing upwards migrations at night (Kristensen, 1983; Bjørke, 2001). The mesopelagic shrimp *Pasiphaea* spp. is widely distributed in the Norwegian Sea and have the highest biomasses around 200–600 m depth (Dalpadado et al., 1998), whereas the shrimp *P. borealis* is known to perform diurnal vertical migrations, ascending in the water column in the evening and returning to the sea bottom in the morning (see Garcia, 2007).

In our study, Greenland halibut larger than 65 cm were the only ones feeding on demersal species such as eelpouts and other flatfish, but they also fed on epipelagic species such as herring. This finding differs from that of previous studies where the diet of large Greenland halibut was dominated by large groundfish, even when smaller size individuals fed heavily on smaller pelagic fish such as capelin (Yang and Livingston, 1988; Bowering and Lilly, 1992; Solmundsson, 2007).

Because their pelagic distribution is supposed to be linked to foraging activity, the presence of suitable pelagic prey should be expected to influence the pelagic behavior of Greenland halibut. Important prey species such as cephalopods, capelin, and herring have strong seasonal migration patterns, and the abundance of these within any given geographic area is prone to seasonal and annual variations. One example would be herring, which was found to be prominent as prey on the continental slope in October 1997, whereas in January 1998 it was not identified as prey at all (Hovde, 2002). Heavy feeding on capelin, another migratory small pelagic species, has also been observed for Greenland halibut (Smidt, 1969; Bowering and Lilly, 1992).

The vertical activity level of individual Greenland halibut showed a clear seasonal pattern, being highest in August–October. This is the period preceding the main spawning season, when mature Greenland halibut gather in the slope area (Albert et al., 2001). It is also the period when annual surveys are made for population monitoring of the Northeast Arctic stock.

Concluding remarks

This is the first published account of adult Greenland halibut being caught in pelagic waters in considerable quantities in any part of its distribution area. In the present study, adult individuals were found to be widely distributed in the water column. Pelagic activity was related to fish size and dial and seasonal cycles and was influenced by variability in the distribution of prey species. Variations in availability to the survey trawl could be biasing the length- and sex-distribution of these catches as well as obscuring trends in population abundance and structure as derived from stock assessment. The methods used in our study are not suitable for quantifying pelagic behavior, but both diet composition and vertical activity from archival tags indicate that use of the pelagic zone may be significant. In order to improve abundance estimates, and thereby the basis for management decisions, it is important to develop methods to estimate the availability of adult Greenland halibut to bottom sampling trawl.

Acknowledgments

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All their eggs in one basket: a rocky reef nursery for the longnose skate (*Raja rhina* Jordan & Gilbert, 1880) in the southern California Bight

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Skates (family Rajidae) are oviparous and lay tough, thick-walled eggs. At least some skate species lay their eggs in spatially restricted nursery grounds where embryos develop and hatch (Hitz, 1964; Hoff, 2007). After hatching, neonates may quickly leave the nursery grounds (Hoff, 2007). Egg densities in these small areas may be quite high. As an example, in the eastern Bering Sea, a site <2 km^2 harbored eggs of Alaska skate (*Bathyraja parmifera*) exceeding 500,000/km^2. All skate nursery grounds have been identified over soft sea floors (Lucifora and García, 2004; Hoff, 2007).

In 2005, while conducting fish surveys using a manned submersible over natural reefs in the Santa Barbara Channel, southern California, we found an area of high skate egg density, located on the edge of Hueneme Submarine Canyon. Until that date, we had rarely observed skate eggs in our southern California surveys. For example, in 362 other submersible dives, in waters between 18 and 365 m deep and encompassing 395 km of transects over a wide range of habitats, we had observed only 44 skate eggs. In 2006, we returned to the Hueneme Submarine Canyon site and examined this nursery ground more closely.

Material and methods

We conducted the study on 24 October 2006 on a feature located on the west side of Hueneme Submarine Canyon (Fig. 1). The study area is a rocky outcrop located at approximately 34°02.3′N, 119°18.1′W. Rocks exposed at the site are likely a submerged extension of the Miocene volcanic rocks that make up Anacapa Island (Vedder et al., 1986). These rocks form gently north-dipping strata of volcanic flows, breccias, conglomerates, or tuffs. Faults and joints observed on the Island, and from the submersible at the site, increase rock resistance against gravitational failure. Bathymetric high spots, such as that of the skate nursery grounds, are north-dipping volcanic-layer outcrops that have not collapsed because of the local strength of the rock substrate in combination with the buttressing structure formed by faulting and jointing. Several diminutive taxa, including squarespot (*Sebastes hopkinsi*), swordspine (*Sebastes ensifer*), and pygmy (*Sebastes wilsoni*) rockfishes, dominate the fish fauna. Among structure-forming invertebrates, the volcanic outcrop also harbors high densities of barrel, flat, foliose, and vase sponges, gorgonian corals, the large anemone *Metridium* sp., basketstars, and the deep-water antipatharian coral *Antipathes dendrochristos*.

This survey was conducted aboard the small (4.8 m in length) research submersible *Delta* (Delta Oceanographics, Ventura, CA). During the dive, we tried to maintain a constant distance within 1 meter of the seafloor and a constant speed between 0.5 and 1.0 knot. The survey was made during daytime hours and we documented the egg density of longnose skates in that area with an externally mounted hi-8 video camera positioned above the middle viewing-porthole on the starboard side of the submersible. The scientific observer conducted a belt-transect survey through this same starboard viewing port, verbally recording onto the videotape all skate eggs observed within 2 m of the submersible.

Navigation fixes (latitude and longitude coordinates) were received from a Thales GeoPacific Winfrog ORE Trackpoint 2 USBL (Fugro-Pelagos, San Diego, CA) system at two-second intervals, and a Winfrog DAT file was generated for the research dive. Distance and duration between fixes were calculated to obtain a point-to-point submersible speed; errant navigation fixes were removed when speed exceeded 2 m/sec. The navigation fixes were then smoothed by using a nine-point moving average, and transect length was

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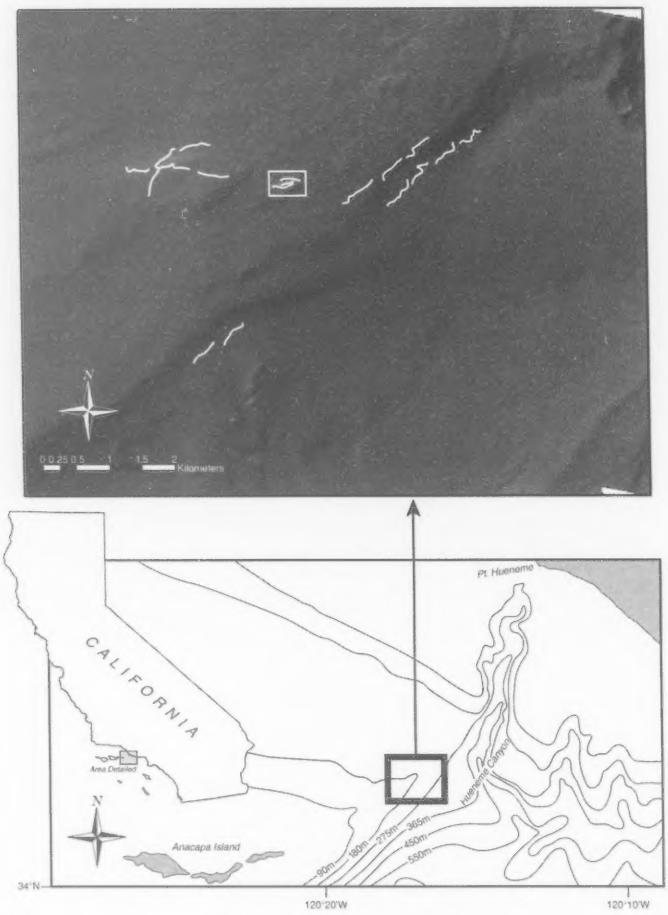

Figure 1

(Lower map) The general location (black box) of the longnose skate (*Raja rhina*) nursery ground on the edge of Hueneme Submarine Canyon, southern California Bight. (Upper map) The area within the black box in the lower map has been enlarged and the white box represents the nursery ground within that area; the other white lines in the upper map denote transects followed by the *Delta* submersible. Transects outside the box did not contain skate eggs.

estimated from the total distance between the smoothed points. Transect length was divided by transect duration to obtain an average transect speed. The length of individual habitat patches was estimated from average speed of the submersible during each transect.

During the survey, the observer verbally noted each egg or egg aggregation, estimated the number of eggs in the aggregation, and whether the eggs were living or dead. We assumed that yellow or green, shiny and full eggs still contained embryos and that those that were dark, deflated, and heavily biofouled were empty. It should be noted that in other studies dark egg cases have been found to still hold living embryos and thus our estimates of viability may be low. At that time, the observer also described whether the egg(s) had been laid on rock, cobble, sand, or on such structure-forming animals as sponges and gorgonians. Lastly, in order to identify the species using this nursery ground, five eggs were collected.

In the laboratory, the abiotic habitats of the nursery ground were also characterized from the survey videotape. We defined these habitats using the different categories of substrata and standard geological definitions defined by Yoklavich et al. (2000). In order

Table 1

Expected and actual (observed) longnose skate (*Raja rhina*) egg numbers by habitat type. Observations were made from the *Delta* submersible, eastern Santa Barbara Channel, October 2006. Habitat types were rock ridge (R), boulder (B), cobble (C), and sand (S). A two-character code was assigned each time a distinct change in substratum type was noted. The first character of the code represents the substratum that accounted for at least 50% of the survey patch, and the second character represents the substratum accounting for at least 20% of the patch. Expected numbers were calculated by assuming that the density of skate eggs was not different across habitat types. Expected numbers were rounded to the nearest integer. Habitats are ordered from most to least area surveyed (m^2).

Habitat	Area surveyed (m^2)	Expected egg numbers	Actual egg numbers
RR	2219.0	1513	1721
RC	208.6	142	13
RB	76.1	52	2
RS	37.6	26	4
CR	10.4	7	0

of decreasing particle size, these substrata were the following: rock ridge (R), boulder (B), cobble (C), and sand (S). A two-character code was assigned each time a distinct change in substratum type was noted. The first character of the code represented the substratum that accounted for at least 50% of the survey patch, and the second character represented the substratum accounting for at least 20% of the patch. Thus, RB represented a patch composed of at least 50% rock ridge and at least 20% boulders.

Results

In the nursery area, we surveyed 2551.8 m^2 of sea floor in waters between 125 and 151 m depth (average depth 139 m) and with bottom temperatures ranging from 9.1° to 10.1°C (Fig. 1). The study site was primarily composed of high-relief rocky ridge (RR), along with lesser amounts of rock-cobble (RC), rock-boulder (RB), rock-sand (RS), and cobble-rock (CR) (Table 1).

We observed 1740 eggs, of which 238 were characterized as intact. As far as we could ascertain, all of the eggs observed were those of the longnose skate (*Raja rhina* Jordan & Gilbert, 1880) (Fig. 2). We observed a range of egg states, from those that appeared to be newly deposited to ones that had almost disintegrated. The eggs did not appear to have been randomly laid over the substrata because significantly more eggs were observed over the highest relief areas (habitat RR) than over the other habitats (chi-square=13.1, df=4, P=0.01) (Table 1). Eggs also appeared to be clumped in their distributions. Although many of the eggs were deposited singly, we

Table 2

Number of observations (from the *Delta* submersible) of each size of longnose skate (*Raja rhina*) egg cluster (no. of eggs in cluster) in the nursery ground on the edge of Hueneme Submarine Canyon, October 2006, categorized by habitat type. Habitat types were rock ridge (R), boulder (B), cobble (C), and sand (S). A two-character code was assigned each time a distinct change in substratum type was noted. The first character of the code represents the substratum that accounted for at least 50% of the patch, and the second character represents the substratum accounting for at least 20% of the patch.

Number of eggs in cluster	Number of observations			
	RB	RC	RR	RS
1	0	13	145	4
2	1	1	38	0
3	0	0	23	0
4	0	0	9	0
5	0	0	6	0
6	0	0	4	0
8	0	0	1	0
10	0	0	6	0
12	0	0	1	0
20	0	0	3	0
24	0	0	1	0
25	0	0	1	0
30	0	0	4	0
40	0	0	2	0
≥50	0	0	8	0
Total	1	14	1722	4

observed several aggregations with 300 or more eggs (Table 2). Most of the eggs were laid on rocks and relatively few on such invertebrates as sponges, deep-water corals, and sea anemones (Table 3). However, the eggs that were laid on invertebrates were significantly more likely to be intact than were those on rocks (intact on invertebrates=95%, 95% confidence interval, 83–99%; intact on rock=12%, 95% confidence intervals 10–13%; chi-square=225, df=1, P<0.0001).

We estimated the total number both of all eggs and only those that were intact. We assumed that our survey transect path demarcated the outer perimeter of the nursery grounds because we noted eggs all along the transect path. Using densities of both all eggs (0.68183/m^2) and only intact ones (0.093 eggs/m^2) and the area bounded by our transects (27,878 m^2), we estimated that there were 19,008 eggs in the nursery grounds, of which 2593 were intact.

Discussion

According to our many years of visual observations, skate nursery grounds are uncommon in southern

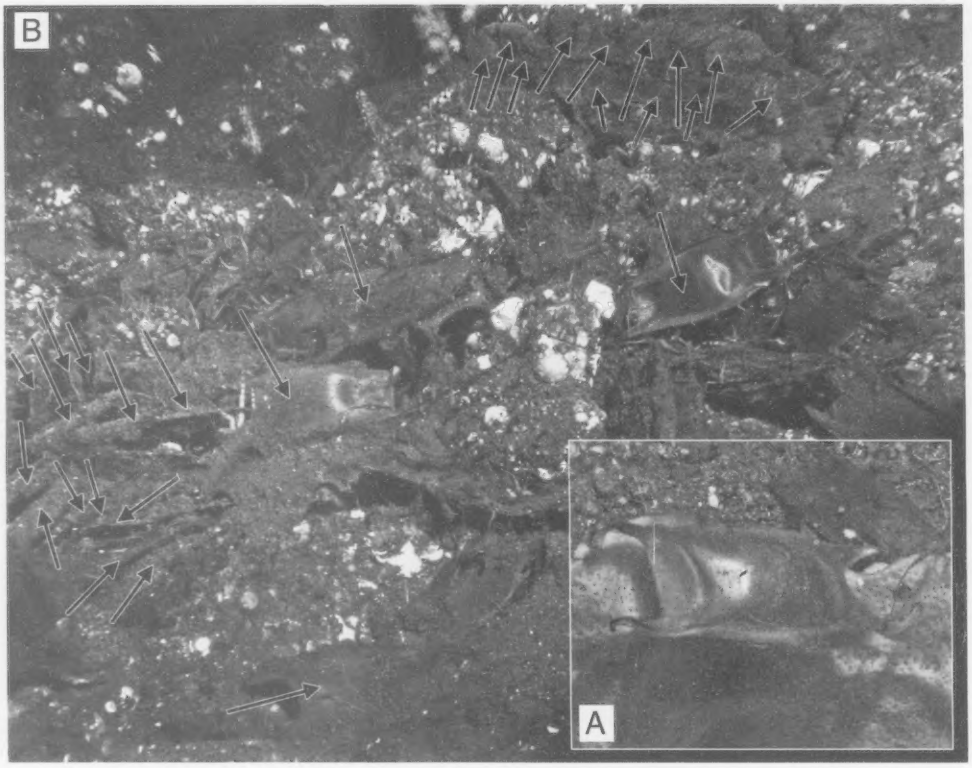

Figure 2

(**A**) An intact longnose skate (*Raja rhina*) egg, (**B**) an aggregation of eggs (see arrows), many of which are covered in fine sediment.

Table 3

Numbers of intact and empty longnose skate (*Raja rhina*) eggs on abiotic (rocks and fishing line) and biotic substrata, observed from the *Delta* submersible, eastern Santa Barbara Channel, October 2006.

Substrata	Number of eggs intact	Number of eggs empty
Abiotic		
Rocks	197	1500
Fishing line	1	0
Total abiotic	198	1500
Biotic		
Sponges	35	1
Gorgonians	3	1
Corals	1	0
Sea anemones	1	0
Total biotic	40	2

California waters (at least at depths <360 m), and like that observed by Hoff (2007) the nursery site that we observed was relatively small in area because visual transects conducted within 1 km over similar habitats yielded no eggs (Fig. 1). However, whereas previously described skate nursery grounds lay on soft sea floors, the study site was a rocky outcrop sitting on the edge of a submarine canyon. This reef appears to be a relatively high-energy area (little sediment was found on the substrata) with high densities of structure-forming invertebrates. Because so few visual surveys have been conducted on the Pacific Coast, we do not know if the nursery ground that we observed was atypical or if there are other such grounds in Pacific Coast waters.

Longnose skates do not randomly lay their eggs over the sea floor. Among the various habitat types in the nursery area, rock ridges contained statistically more eggs and these eggs were often found in clumps. It would be expected that eggs laid on the highest relief would be most exposed to currents and arguably less

available to at least some predators than eggs lying on low substrata.

Skate reproduction, with its emphasis on highly spatially restricted nursery grounds, appears to be an example of "predator swamping" (Van Montfrans et al., 1995). In addition, the observation that intact skate eggs are more likely to be found on sponges than on bare rocks may reflect an antipredator **strategy** by adult skates. Boring snails, including those in the families Muricidae, Naticidae, and likely Ranellidae, are major predators on skate eggs and predation rates in nursery grounds can reach 40% or more (Lucifora and García, 2004; Hoff, 2007). Snails of all of these families are found in southern California waters (McLean and Gosliner, 1993). Although they could not identify them in their trawl-based study, Lucifora and Garcia (2004) speculate that microhabitat differences in egg placement could lead to variable protection from predators. It is possible that sponges, with their spicule-rich skeletons and potent chemical defenses, may deter snails or other egg predators.

The Bering Sea skate nursery ground that Hoff (2007) investigated was notable for having 1) high currents and productivity and 2) the presence of only mature fish. The nursery site of our study was composed of high-relief rocks situated on the edge of a submarine canyon. Because of the relative absence of fine particulates on the substrata and the presence of unusually high densities of sponges, gorgonians, and other structure-forming invertebrates, we surmised that this nursery ground is bathed by high currents and is likely quite productive. In addition, we observed no juvenile or adult skates, providing additional evidence for Hoff's (2007) hypothesis that newly hatched skates quickly leave their nursery grounds.

In the context of this nursery ground much about longnose skate reproduction remains unknown. For instance, we do not know when the eggs are laid, their incubation period, nor the fate of the juveniles. We do not know how many skates use this habitat and how they migrate. Lastly we note that the extent to which female skates seek out sponges is unknown. However, if skate eggs deposited on sponges are at a competitive advantage, damage to these invertebrate communities (i.e., through destructive fishing practices) and the subsequent increase in egg predation, would have a detrimental effect on skate reproductive success.

Acknowledgments

D. Ebert identified the skate eggs and J. Hoff provided a great deal of useful background information on skate nursery grounds and provided us with a copy of his Ph.D. thesis. Also very useful were the discussions with D. Cadien on sponge and snail ecology. We thank the pilots of the research submersible *Delta*, J. Lilly and C. Ijames, and the crew of the research vessel *Velero* for support in the field.

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Microsatellite primers for red drum (*Sciaenops ocellatus*)

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In this note, we document polymerase-chain-reaction (PCR) primer pairs for 101 nuclear-encoded microsatellites designed and developed from a genomic library for red drum (*Sciaenops ocellatus*). Details of the genomic library construction, the sequencing of positive clones, primer design, and PCR protocols may be found in Karlsson et al. (2008). The 101 microsatellites (GENBANK Accession Numbers EU015882-EU015982) were amplified successfully and used to genotype 24 red drum obtained from Galveston Bay, Texas (Table 1). A total of 69 of the microsatellites had an uninterrupted (perfect) dinucleotide motif, and 30 had an imperfect dinucleotide motif; one microsatellite had an imperfect tetranucleotide motif, and one had an imperfect and compound motif (Table 1). Sizes of the cloned alleles ranged from 84 to 252 base pairs. A 'blast' search of the GENBANK database indicated that all of the primers and the cloned alleles were unique (i.e., not duplicated).

Summary genotypic data, based on 22–24 assayed red drum, also are given in Table 1 and include number (and size range) of alleles detected, observed and expected heterozygosity, and probability values from tests for conformity to Hardy-Weinberg equilibrium expectations. One microsatellite (*Soc*734) was monomorphic; the number of alleles detected at the remaining 100 (polymorphic) microsatellites ranged from 2 to 26. Estimates of observed and expected heterozygosity and tests for conformity to Hardy-Weinberg and genotypic equilibrium expectations were performed with GENEPOP (Raymond and Rousset, 1995). Observed heterozygosity (polymorphic microsatellites) ranged from 0.042 (*Soc*706) to 1.000 (11 microsatellites, Table 1) and averaged (±standard deviation [SD]) 0.775 ±0.211; expected heterozygosity ranged from 0.042 (*Soc*706) to 0.971 (*Soc*636) and averaged 0.806 ±0.201. After Bonferroni correction (Rice, 1989), genotypes at 99 of the polymorphic microsatellites did not differ significantly from Hardy-Weinberg equilibrium expectations. At one locus, *Soc*706, there were only two alleles, one of which was observed only in a heterozygote; this microsatellite was not tested for Hardy-Weinberg equilibrium. Analysis with MICRO-CHECKER (Van Oosterhout et al., 2004) indicated the possible occurrence of null alleles at nine of the microsatellites, and single base-pair shifts (i.e., alleles differing by only a single base pair) were observed at five of the microsatellites (Table 1). Tests of genotypic disequilibrium were nonsignificant after Bonferroni correction. Given that red drum possess 24 haploid chromosomes (Gold et al., 1988), several of the microsatellites undoubtedly are linked; determination of linkage will await formal mapping studies.

Along with PCR primers for red drum microsatellites developed previously by O'Malley et al. (2003), Saillant et al. (2004), and Karlsson et al. (2008), the primers developed here will be useful in a variety of applications (Liu and Cordes, 2004), including analysis of stock structure, monitoring and assessment of red drum stock enhancement, parentage analysis as employed in aquaculture, and the generation of a genetic map for red drum. A table of the 269 PCR primers developed for red drum may be found at <http://wfsc.tamu.edu/doc> under the file name "PCR primers for red drum (*Sciaenops ocellatus*) microsatellites."

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Table 1

Summary data for 101 microsatellites developed for red drum (*Sciaenops ocellatus*). Primer sequences are forward (top) and reverse (bottom) primers used in PCR amplification of both DNA strands. Repeat sequence indicates repeat motif; subscript indicates number of replicates in the cloned allele. Cloned allele is size (in base pairs) of the sequence in GenBank. N is the number of individuals assayed; N_A is the number of alleles detected. Size range refers to size (in base pairs) of alleles thus far detected and size includes the 21 base pair 5′ tail-sequence primer used for PCR amplification. H_O/H_E is observed and expected heterozygosities, respectively. P_{HW} represents the probability of deviation from Hardy-Weinberg expectations.

Micro-satellite	Primer sequence (5′–3′)	Repeat sequence	Cloned allele	N	N_A	Size range	H_O/H_E	P_{HW}
*Soc*628	GCACTGCTGCTACCTGACAC TCCAACTCACTCGTTGTTTACAG	$(GA)_{29}$	181	24	18	174–220	0.917/0.932	0.783
*Soc*630	CGCTGCTTCTCACGCTCTG GGTGTCATGTATGGATACTGCTCTT	$(CA)_{16}$	144	24	10	155–175	0.833/0.841	0.357
*Soc*631	TGGGTCCTCCAGCTCTGATA TCTGTTTCCATTGCTGTGTCA	$(CA)_{10}(N)_{22}(CA)_{14}$	222	24	22	189–241	1.000/0.965	1.000
*Soc*632	TCTTTTCTCTGCGTTTGATAG TCTCTGTGTTTTCGCTTCCA	$(CA)_{12}$	242	24	2	262–264	0.083/0.082	1.000
*Soc*633	CAAATGACAAGGAAGACAAGAGC CCGCCTCAGTTACAGGAAATG	$(GA)_{18}(N)_2(GA)_5$	188	24	8	191–217	0.875/0.830	0.277
*Soc*635	CATCAGCACGGTTATTTTCTTG CCTCTCTCTTTTCTTCCCTCG	$(CA)_{23}$	245	24	10	249–269	0.708/0.781	0.609
*Soc*636	AACACGTAACAACATCCATG GATCAAATTAGCATCAGTAATGT	$(CA)_{35}$	150	23	25	131–217	0.957/0.971	0.134
*Soc*637[1]	AGAGGGTTAGAAGGGGAGAA AGTATCAAAACTTGGCATCC	$(CA)_{31}$	194	22	20	178–230	0.818/0.940	0.007
*Soc*638	CTTTCATTTGGACTGGCTTTG TGGTCTGCTCTGCTTTTGATT	$(GA)_{14}$	220	24	5	236–250	0.792/0.755	0.323
*Soc*639	ATCTTCTCTCACAAACACTCAC CCAAACTGTCAAGGATGTCA	$(CA)_{13}$	138	24	7	148–168	0.792/0.740	0.989
*Soc*640	AGGACATTTGGAGTGGAAGAGTA ATGGGGACAGGAGGTTTTCTA	$(CA)_{14}$	159	24	13	179–209	0.875/0.898	0.225
*Soc*641	ATGCGGAGTAGAGAGAGGAGG TGACCTGAATGAAGATGTATGGA	$(CA)_{17}(N)_2(CA)_6$	136	23	20	142–202	0.913/0.958	0.117
*Soc*642	CAGAGAAGGTTACAGGAGGTG ATGTGCTGGGACAGGTTG	$(CA)_{22}$	200	23	9	210–228	0.870/0.881	0.539
*Soc*644	GCTACGGTCGGGAATCAGA GCTGCCCTTCAGTGGTTTT	$(CA)_{32}$	167	23	18	155–205	0.913/0.952	0.319
*Soc*645[1]	GAGTTGGTCAATAGCCACAGG ATCTGAAGGGCAGGTGTTTG	$(CA)_{20}$	150	24	10	157–181	0.708/0.891	0.025
*Soc*646	GGGAAAGTAGATAGGGGCACA AGAGGTCAGGGTTGAGCAGAGT	$(CA)_{16}$	132	22	4	130–156	0.273/0.318	0.559
Soc647	GCACAGATTCGTCCCAGATT TGTTCAGTTGCTCACAAACCA	$(CA)_{51}$	247	23	26	203–283	0.957/0.967	0.463

continued

Table 1 (continued)

Micro–satellite	Primer sequence (5′–3′)	Repeat sequence	Cloned allele	N	N_A	Size range	H_O/H_E	P_{HW}
*Soc*648	GCTTCCAGATGCGATGTAAAC GCTACTGCCACCACAAACAC	$(CA)_5(N)_2(CA)_{22}$	181	24	14	179–225	0.833/0.888	0.266
*Soc*649[1]	GTACTTGTAGTAATCAGGGTATTTT AAACGCACTTCTTTGAGACTA	$(TG)_{20}$	154	24	11	152–182	0.708/0.878	0.040
*Soc*650[1]	CCGTGTTTGCTTGGCTTT CAATGTGTATGGGTATGGGTAGG	$(CA)_{22}$	125	24	14	127–175	0.583/0.893	0.002
*Soc*651	TGAAAGACGAAATTCAGCAAAA AGGAAGGCAGGTGGATAGTAAA	$(CA)_{23}$	187	24	20	193–263	1.000/0.931	1.000
*Soc*652	TCGCCATCATTCATAAAACT GTTCTCAAAAGAGTCTACCTGTC	$(CA)_{19}$	196	24	10	204–232	0.833/0.785	0.821
*Soc*653[1, 2]	GAAACAGCCTCCCACACAG TCCTCCTCTTCGCTCATCA	$(CA)_{26}$	252	24	17	138–274	0.792/0.931	0.157
*Soc*654[2]	CTCCGCTGCCAAACTGAC TGTGCTCTACATCCTCCTCCT	$(CA)_{13}$	164	23	5	173–186	0.565/0.575	0.191
*Soc*655	CTGAAAGGGCCGTTGGTTT GAGGATGCAGAGTCAAGCAAGA	$(CA)_{18}(N)_2(CA)_3(N)_2(CA)_8$	166	24	10	172–192	0.792/0.836	0.642
*Soc*656	CGTGCTGAGACAGGTGGTAGT GTAGGCGTGGAGACGGAGT	$(CA)_{24}$	213	24	23	221–279	1/0.955	0.442
*Soc*657	GGAAAGCAAAGCAAAAGAAACT AGCCGAATGAGACAGAGGAAA	$(CA)_{34}$	232	22	17	210–268	0.955/0.946	0.757
*Soc*658	AATCTCCCAGTGCCTTTGA CTGCTTTTTCCCTCTATTTCTC	$(GA)_{14}$	149	24	7	162–176	0.542/0.558	0.789
*Soc*659	GCATCCCTCCCCTCTCTCC GCCTGGCAAACATCCAACTT	$(CA)_{17}$ $(CA)_{12}(N)_2(CA)_2(N)_2(CA)_5$	189	23	10	200–228	0.870/0.854	0.462
*Soc*660[2]	TTGCCAATGTTCTTTCTCTCT ATTCCTACTCCTGCCAAGAT	$(N)_2(CA)_2(N)_2(CA)_7$	123	24	11	130–184	0.833/0.847	0.783
*Soc*661[1]	ACCGCCTCAAAACAACACA AGGAGATTGGGAGTGGAGATA	$(CA)_{13}$	145	24	11	154–176	0.667/0.859	0.004
*Soc*662	CGTCTTTAGGAAGGTGTGGC CCTGTCTGGAGGGGAAAAC	$(CA)_{20}(N)_2(CA)6(N)_2(CA)_3$	108	22	13	90–144	0.818/0.910	0.385
*Soc*663	TCAGGGTATGTACGCAGATG CAAGCACTTCACGAGGAAC	$(CA)_{14}$	172	24	21	190–274	0.917/0.930	0.753
*Soc*664	GAGGTTCAGTTTGGCTGCTG CGTGTGTGTTTTCGCTCAGTT	$(CA)_{23}$	136	24	15	137–169	0.875/0.907	0.268
*Soc*665	TCACAGTGGCTCTCCAGGTAA TTGCCCTCTTGTCGTTCATCT	$(GA)_{33}$	132	24	22	115–189	0.833/0.944	0.268
*Soc*666[1]	TAATCTCTGTGTGTGTCCAGGTG GACGCAAGGCTGAGGCATA	$(CA)_4(N)_2(CA)_{21}$	201	24	18	207–263	0.750/0.921	0.020

continued

Table 1 (continued)

Micro–satellite	Primer sequence (5′–3′)	Repeat sequence	Cloned allele	N	N_A	Size range	H_O/H_E	P_{HW}
*Soc*667	TAACGCTCTGTCCATCACTG CATCTACGAATGCCCAACA	$(CA)_{14}(N)_4(CA_{)5}$	232	23	6	245–261	0.652/0.699	0.198
*Soc*668	TGATGAGGACCAAGTGACAAC ATCAGTGTTTTTCCATTAGCC	$(CA)_{18}$	96	23	15	107–163	0.870/0.901	0.095
*Soc*669	GAAGGAGGCAGGCACACATA GAGAGCGAGCGAGAGAAAGA	$(CA)_4(N)_2(CA)_{21}$	117	23	17	115–167	0.957/0.889	0.996
*Soc*670	TTCGTCCCGTCACAGCACA CAAAGAGAGATGAATAAACCCAAAG	$(CA)_{30}$	245	23	12	225–273	0.826/0.852	0.296
*Soc*671	CGCCTCTCTTCCTCAGATGT ACAGTGGGCAAATCCATACA	$(CA)_{30}(N)_4(CA)_4$	228	23	14	233–271	0.826/0.910	0.152
*Soc*672	CGTATGGTGAGTGTTGGCA TGTCGTCTCTGAATGTGTCCT	$(CA)_{22}$	199	24	19	199–243	0.875/0.946	0.056
*Soc*673	AAAGACTGACACAAGGCTGACA AATCTCTGCTCATTTCCTCATCT	$(CA)_{24}$ $(CAGA)_4(N)_4(CAGA)_2(N)$	194	22	10	193–227	0.773/0.819	0.807
*Soc*675[1]	TGTCCCCATAAAGAACAAGG ACACAACGTCTACAGGAAGGC	$(CAGA)_2$	96	24	6	108–234	0.417/0.608	0.037
*Soc*676	TGAACTGTCACGTCCGTCAT CCTTGTTCTTTATGGGGACA	$(CA)_{20}$	166	24	15	173–211	0.875/0.919	0.320
*Soc*678	CTGGCTGGTTGATGTAAGTC AGACAGTGGGGCGTTAGAT	$(CA)_{19}$	128	24	12	132–158	0.875/0.898	0.577
*Soc*679	ACACCTTCCACTGACTGACCAC GAATGTGTGCGTGTCTGCGT	$(CA)_{27}$	146	24	14	162–194	0.792/0.889	0.013
*Soc*680	GCTGCCTCCTCTGTCACTCT TCACGCACCTTCTCCTCTTT	$(CA)_{12}$	93	22	19	106–152	0.955/0.949	0.632
*Soc*681	TATGGCTCCGACACACTCCT CCACCCCAGTAGACACTCAGA	$(GACA)4(CA)_{12}$	111	24	9	123–151	0.875/0.849	0.671
*Soc*682	TCCACTGTAGGTGTTGTTTCA ACTTTTAGGCGGGAGAGTC	$(CA)_{15}$	200	24	11	202–236	0.750/0.880	0.171
*Soc*683	TTCGCACACATACATAACTAAACT AGCGTCATAATCCAACTGTCA	$(CA)_6(N)_2(CA)_7$ $(GA)_5(N)_8(GA)_4(N)_2(GA)_2$	185	23	7	190–230	0.565/0.647	0.312
*Soc*684	CAGCCATACAGACGGACAC TTTGAATAGAAGTCAGAAGTGC	$(N)_2(GA)_6(N)_2(GA)_9$	198	24	13	185–233	0.917/0.833	0.860
*Soc*685	TCAAACAGGGTCATTGGTGA AGGAGAAACGCAGGGAAGA	$(CA)_{14}$	215	23	2	233–235	0.261/0.232	1.000
Soc686	AAAGTGACCCTGAGTTCCTCTG GCAAAAACAATGAAACCCTCT	$(CA)_{19}$	226	23	12	233–265	0.826/0.881	0.564
*Soc*687	GCTGGAAGGGGAGTCTTATGA AAGGCTCTCTCAAGCAGTGTT	$(CA)_{12}$	157	23	16	172–206	0.870/0.928	0.351

continued

Table 1 (continued)

Micro–satellite	Primer sequence (5′–3′)	Repeat sequence	Cloned allele	N	N_A	Size range	H_O/H_E	P_{HW}
Soc688	GCTCTCTTGTCCTCCATACCTG GGGAAACCTGCTGTGACCA	$(CA)_{18}$	192	24	15	198–242	0.875/0.854	0.901
Soc689	GACTCTTCAATCCTACTCACCA ACATTTCTTTTGTCCGTTCA	$(CA)_{25}$	182	23	20	204–246	1.000/0.937	0.951
Soc690	CGTCTTCTGTGTTTGTGGGA TGATTCATCTGCGTCGTGTT	$(GA)_{21}$	101	22	11	109–139	0.909/0.862	0.100
Soc691	CAGTGAGAGACGCAAACCAG GTCAAGTAGGTAAATAAAGCCTGA	$(CA)_{31}$	129	22	15	112–158	0.909/0.926	0.832
Soc692	TGCTGCCATTGAGAAGAGA TTTGTATGTTAGGGGTTGTGT	$(CA)_{10}$	117	23	6	126–140	0.652/0.647	0.650
Soc693	AAGTTGTTTCTTTTGTTCCAGTA TAGATGAAGGCTTGTTTTTCTCT	$(GA)_{18}(N)_1(GA)_7$	192	24	22	279–337	1.000/0.950	0.022
Soc694	CTCGCTCCCATCGTGACT TCCTGAAAGTTGTGCTTGTCC	$(CA)_{11}$	168	21	9	185–207	0.810/0.846	0.424
Soc695	TCTGGAGGGATGATGTGTTT CCTGTTTCACTGCTACTCGC	$(CA)_{21}(N)_2(CA)_5$	135	23	16	129–187	0.957/0.912	0.780
Soc696	GAAAATGGTGAAAACCCTGA CAAAATGGAGAAGCCTGAAG	$(CA)_{31}$	207	23	21	191–253	1.000/0.954	0.937
Soc697	AAGCACGCAATCTATGAAACAC TGTGGGAAAACATCCAGAGG	$(CA)_{10}$	166	22	7	183–199	0.682/0.744	0.421
Soc698	TCTCAAATCTTCCAATCAGCAG CGACAGGGAGTGACCAACAG	$(CA)_{11}$	133	22	9	152–172	0.818/0.819	0.684
Soc699	AGCCAGCAACTGAATACCA CAAAGCCACCTCCTGATTT	$(CA)_{13}$	163	23	15	170–224	0.783/0.853	0.218
Soc700	GCTGAACTGTGAGGCAGATGA CGTAAATAAGGGAAACAAACAAGA	$(CA)_{28}$	245	22	22	249–305	0.955/0.946	0.701
Soc701	ATCCAGGTTTAGAGGCTGTCA TTCAGGAAAAGTCCACACAGA	$(CA)_6(N)_2(CA)_{15}$	120	23	12	129–169	0.739/0.846	0.034
Soc703	TCTCCGTGTAAAGATGATGGTC GAGGGCGAACAAACTGCTAA	$(CA)_{18}$	235	23	16	236–278	0.913/0.923	0.910
Soc704	CCCAGCGTTTCCCCTAAT ACATCCCCTCTGTTCCTCCT	$(GA)_{15}$	237	22	12	254–302	0.682/0.722	0.303
Soc705[2]	CTGCCTGGATTGCCTTTTAG TTCTGCTGTTGAGGACGATG	$(CA)_7(N)_{28}(CA)_4$	215	23	9	235–246	0.826/0.752	0.755
Soc706	ACTCTGTTGCTCCACTACCCA GCTCTTCTCCTGTTTGTGTGA	$(CA)_5(N)_2(CA)_8$	150	24	2	170–176	0.042/0.042	na
Soc707	TTCCTCTCTTCACTCCTGGT CAACACTGCCTCCCTATGG	$(CA)_{21}$	156	23	18	173–217	0.870/0.932	0.287

continued

Table 1 (continued)

Micro–satellite	Primer sequence (5′–3′)	Repeat sequence	Cloned allele	N	N_A	Size range	H_O/H_E	P_{HW}
*Soc*708	TTCCCACTAGAGCTGTGATTGA TCTGACTTCCTCTGCCCATT	$(CA)_{17}$	130	23	9	139–157	0.870/0.878	0.258
*Soc*709	CTGATGAATGGACTGAACAC TGTGAGAGGAACAGAGACG	$(CA)_{22}$	200	22	7	201–221	0.682/0.740	0.261
*Soc*710	TGTGTGAGTGAAAGGCAAACC TGGCAGGGAGAATAGAGGAA	$(CA)_{18}(N)_2(CA)_4$	102	22	10	107–131	0.591/0.674	0.033
*Soc*711	CCTCCCCACCCTCTCTCTG AGTCGCTGCTGTGTGTTTGT	$(CA)_5(N)_2(CA)_{14}$	244	23	10	262–288	0.783/0.807	0.552
*Soc*712	CAGCAGAGGGCAAAAATGA AGGGTGGGTGGTGTTGTCA	$(GA)_{14}$	84	23	7	95–111	0.652/0.557	0.836
*Soc*713	AATAGTTTCCTCTGGATTGACG TGGCTTAGACAAGTGGTGCT	$(CA)_{18}$	189	24	11	189–211	1.000/0.888	0.919
*Soc*715	TTTCCCTCTCTTTCCCACAG GCAACACAGTCAGCCCACA	$(CA)_{10}$	98	24	4	114–124	0.583/0.666	0.716
*Soc*716[2]	ATGGTGTTTGGCTTGCTGAG AGAGCGGCAGTCATCTGTTC	$(CA)_{10}(N)_2(CA)_6$	144	24	15	146–186	0.833/0.921	0.040
*Soc*717	ACGGCAACTGGGGTCAAC TTCCATCGTGTAGGTCACAAAC	$(CA)_6(N)_2(CA)_5$	241	22	3	260–264	0.636/0.609	0.818
*Soc*718	CCCTCTGTTTTCTCTGGGATTT CTTTGGTGTGTGCGTGTTTCT	$(CA)_{16}$	190	23	9	203–223	0.783/0.796	0.041
*Soc*719	CACCTTCTACTCCCCATTCA CCTCCTTCTTGGCTTCATA	$(CA)_{27}$	183	24	17	190–230	1.000/0.917	0.397
*Soc*721	GGTAAAGTGTAAAATGCCAATAG AGGGAAACGAAAAGAGGAAA	$(CA)_{10}(N)_{24}(CA)_{12}$	203	23	10	209–229	0.783/0.847	0.142
*Soc*723	CAGATGGCACTAACTAAGGAGA CAGATAAGTAGAGGTGGAAGACA	$(CA)_7(N)_2(CA)_{24}$	170	24	19	183–245	0.833/0.940	0.171
*Soc*724	GTATTTTGAGCCTGCGTTGTA CATGAACCCGTCTATTTGC	$(CA)_{22}$	194	24	11	198–234	0.833/0.892	0.021
*Soc*725	GAAGCGAAAATAAGGCTGA GTTTAGTTTGGTCTGTTCAATC	$(CA)_{15}$ $(CA)_{15}(N)_2(CA)_4$	88	22	9	101–123	0.773/0.841	0.088
*Soc*726	GCTTTCTATTGATTCTGCCTGTC CAAGGTGGGGAGGAGAGACA	$(N)_2(CA)_{11}$	146	24	16	140–184	0.958/0.918	0.270
*Soc*727[1]	AGGCATCATTTACACGCTCA AACCAACACACGAGGCTGA	$(CA)_{17}$	138	22	8	136–164	0.591/0.779	0.071
*Soc*729	CTTCTTCCACCTGTAACTGAATC CTTTGTCCTCCGCTTGTTTC	$(CA)_9(N)_2(CA)_4$	137	23	13	156–184	0.913/0.930	0.810
*Soc*730	GCACAGGGAGATAAACACAG CTGAAGAAAAGCCAGAGTGAA	$(CA)_{14}$	92	24	4	109–115	0.333/0.487	0.019

continued

Table 1 (continued)

Micro–satellite	Primer sequence (5′–3′)	Repeat sequence	Cloned allele	N	N_A	Size range	H_O/H_E	P_{HW}
Soc731	CAACCGATGAGAGCAGAGACT TGTGTGTAGGTGAGACAAAAGAA	$(CA)_{22}$	224	23	9	220–254	0.391/0.484	0.133
Soc733	GCTGATGATGAACTGGTGCT TTTCTTGTATGCTCTGTTTTGC	$(CA)^{12}$	158	23	3	176–188	0.130/0.127	1.000
Soc734	CAGATTGGAACTGACACACAC AAGCCCCTTCTTGGAGATT	$(CA)_{10}(N)_2(CA)_3$	90	22	1	109	0/0	—
Soc735	CCATCAGGAGGCTTTTGTCA AAGAAGTAGGGTCAGCACACTGT	$(CA)_{31}$	187	23	23	164–244	1.000/0.965	1.000
Soc736	GAACCACCACTGCGAAACT GCCTACAGATTGAGAACCACT	$(CA)_3(N)_2(CA)_9(N)_2(CA)_6$	168	22	3	188–196	0.136/0.132	1.000
Soc737	TCCAGGAAGTGGTCGGTTT GGGAGAATGTGTGAATGCTTG	$(CA)_6(N)_2(CA)_{19}$	197	22	12	203–247	0.818/0.869	0.242
Soc738	TGTAACAGCAGAGACTGAAGC CTGGGTGAAAGGCAGAGTA	$(CA)_{28}$	117	23	21	116–170	1.000/0.949	0.651
Soc739	TTGTGTTTTTCCCTCTGCTG TGTTGTTTATGTTGGTGTGGTG	$(CA)_3(N)_2(CA)_3(N)_2(CA)_{10}$	188	23	16	201–251	1.000/0.938	0.894

[1] Microsatellites where occurrence of null alleles was indicated by analysis with MICRO-CHECKER

[2] Microsatellites with observed alleles differing by only a single base

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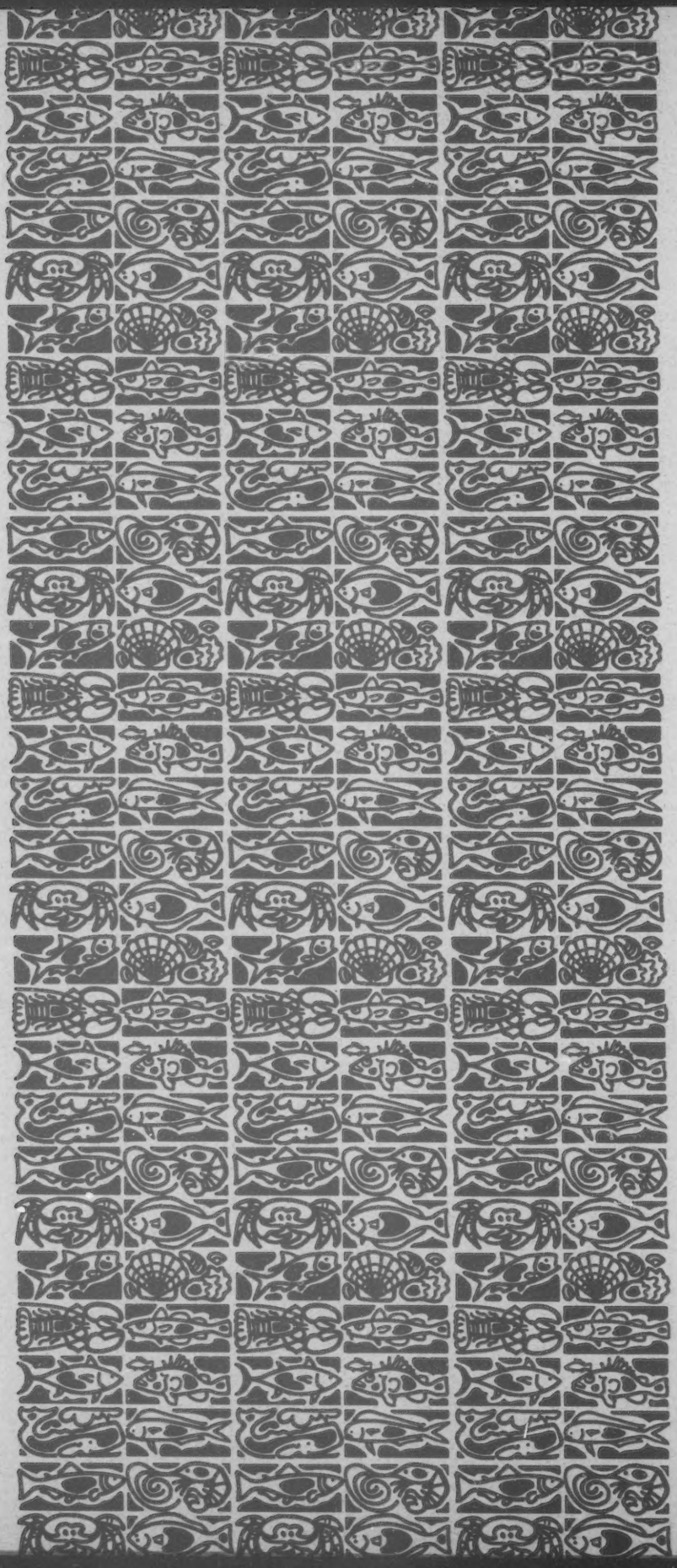
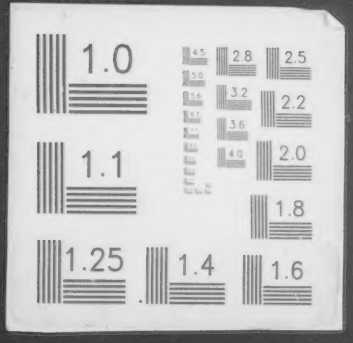
This statement is required by the Act of August 12, 1970, Section 3685, Title 39, U.S. Code, showing ownership, management, and circulation of the *Fishery Bulletin*, publication number 366-370, and was filed on 22 August, 2008. The *Bulletin* is published quarterly (four issues annually) with an annual subscription price of $36.00 (sold by the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402). The complete mailing address of the office of publication is NMFS Scientific Publications Office, NOAA, 7600 Sand Point Way NE, Seattle, WA 98115. The complete mailing address of the headquarters of the publishing agency is National Marine Fisheries Service, NOAA, Department of Commerce, 1315 East-West Highway, Silver Spring, MD 20910. The name of the publisher is Willis Hobart and the managing editor is Sharyn Matriotti; their mailing address is: NMFS Scientific Publications Office, 7600 Sand Point Way NE, Seattle, WA 98115. The owner is the U.S. Department of Commerce, 14th St. N.W., Washington, DC 20230; there are no bondholders, mortgages, or other security holders. The purpose, function, and nonprofit status of the organization (agency) and the exempt status for Federal income tax purposes has not changed during the preceding 12 months. The extent and nature of circulation is as follows: total number of copies (A) (average number of copies of each issue during the preceding 12 months) was 1038 and the actual number of copies of the single issue published nearest to the filing dates was 1034. Paid circulation (B) is handled by the U.S. Government Printing Office, Washington, DC 20402, and the total number printed for sales (mail subscriptions and individual sales) was 225 for the average number of copies each issue during the preceding 12 months and 225 the actual number of copies of the single issue published nearest to the filing date (C). Free distribution (D) by mail; samples, complimentary and other free copies (average number of copies each issue during the preceding 12 months) was 773 and the actual number of copies of the single issue published nearest to the filing date was 769. Free distribution outside the mail (E) by carriers or other means was 0 for both average number of copies and actual number of copies. Total free distribution (F) was 0 for both average number of copies and actual number of copies of the single issue published nearest the filing date. The total distribution (G: sum of D and B) (average number of copies each issue during the preceding 12 months) was 1016 and the actual number of copies of the single issue published nearest to the filing date was 994. There were 40 copies (avg. annual) not distributed (H). The total (I: sum of G and H) is equal to the net press run figures shown in Item A: 1038 and 1034 copies, respectively. I certify that the statements made by me above are correct and complete: (Signed) Willis Hobart, Publisher.

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